J. Pharm. Pharmacol. 1999, 51: 891–898
Received December 8, 1998

Cancer Chronotherapy

FRANCIS LÉVI

Laboratoire Rythmes Biologiques & Chronothérapeutique, Hôpital Paul Brousse, 94800 Villejuif, France

Abstract

The cytotoxicity of more than thirty anticancer drugs varies by more than 50% as a function of dosing time along the 24-h time-scale in laboratory animals. Mechanisms involve circadian changes in cellular metabolism and proliferation processes, as well as drug pharmacokinetics. Moreover, the administration of chemotherapy at the least toxic time usually achieves best antitumour efficacy in experimental tumour models. Here we review experiences in utilising these phenomena in the optimization of cancer chemotherapy in the clinic.

Chronotherapy has been administered to 1500 patients with metastatic colorectal cancer using 5-fluorouracil and leucovorin with or without oxaliplatin. Sinusoidal chronomodulated delivery of 2- or 3-drug chemotherapy was performed in the patient's home or during usual activities, with a computer-programmed multi-reservoir pump. Courses lasted 4–5 days and were repeated every 14–21 days. Three-drug chronotherapy proved largely superior to flat infusion with respect to both tolerability and antitumour efficacy. The better tolerability of chronotherapy further allowed an increment of both 5-fluorouracil and oxaliplatin doses, which in turn further improved objective tumour response rate to 66%. This enabled surgical removal of previously inoperable metastases and the achievement of $> 20\%$ survival at three years.

Second generation programmable-in-time pumps have simplified chronotherapy administration and decreased its costs. A broad use of fully ambulatory chronotherapy requires thorough definitions of drug stability, and compatibility with pump reservoirs and other medications.

Circadian Rhythms

Biological functions of living beings are organized along a 24-h time scale. These circadian rhythms are endogenous, since they persist in constant environmental conditions. This property has been demonstrated in microorganisms, such as *N. crassa*, in plants and in all kinds of animal species, such as flies, mice, rats and humans. Usually, the endogenous period, which is the duration of the cycle, slightly differs from precisely 24 h. In man, the average endogenous circadian period is close to 25 h, with interindividual variations however (Touitou & Haus 1992).

The endogenicity of circadian rhythms can be accounted for by specific genes. For instance, mutations of the *per* gene in Drosophila or the *clock* gene in mouse result in severe disturbances of the rest–activity circadian cycle and other rhythms. These alterations can consist in a shortening, a lengthening or even a suppression of the circadian period, according to the kind of mutation and to the environmental conditions (Vitaterna et al 1994; Plautz et al 1997). Studies performed in homozygous or heterozygous human twins further support the role of a genetic basis for circadian rhythms in man. Indeed, the recent identification of *per* homologues in human and rodent cells suggest the ubiquity and similarity of the genetic control of the circadian organization in various species (Tei et al 1997). Thus, a general circadian clock mechanism was described by a negative feed-back loop involving transcription and translation of the circadian genes (Green 1998).

External cycles reset the endogenous rhythms of mammals through a circadian system, which

involve the suprachiasmatic nucleus (SCN) and the pineal gland, among other structures (Klein et al 1991). The SCN plays a key role in this organization. Thus, the physical destruction of the SCN in rodents results in a complete suppression of several rhythms, such as the rest–activity cycle and the body temperature rhythm, and SCN transplantation restores these rhythms.

The regular alternation of light and darkness over 24 h is a potent synchronizer of the circadian system. It calibrates the endogenous period to precisely 24 h through the effects of light and melatonin, a hormone mostly secreted by the pineal gland during darkness (Klein et al 1991; Touitou & Haus 1992). Under such synchronization, mammals with normal circadian function display circadian rhythms in cellular metabolism and proliferation, with predictable amplitudes and times of peak and trough. These rhythms influence anti-cancer drug pharmacology and ultimately tolerability and antitumour efficacy of cancer treatment (Lévi 1997). Conversely, a lack of synchronization, a deficiency in its perception, or an alteration of circadian clock function make rhythm peaks and troughs unpredictable, and may require specific measures for chronotherapy to improve the therapeutic index (Deprés-Brummer et al 1998).

The rest–activity cycle is one of the most obvious rhythms. Its endogenicity has been demonstrated as it persists in constant environmental conditions in flies, rodents and humans. This rhythm is controlled by the *per* gene in Drosophila and by the *clock* gene in mouse. Direct pharmacologic actions targeted at the SCN level in rodents can modify the timing of biochemical or molecular rhythms in these nuclei. These changes translate into a phase shift of the rest–activity cycle of these animals. These and other experimental findings (see above) clearly demonstrate the dependency of this rhythm upon SCN function. The easy recording of this rhythm has further supported its use as a reference rhythm for circadian timing of medications and more recently for assessing circadian system function.

Implications of Rhythms for Cancer Therapy: Group Chronotherapy

Experimental and clinical prerequisites

Chronopharmacology of anticancer drugs in laboratory rodents. The circadian time of administration of over 30 anticancer agents influences the extent of toxicity and anticancer activity in mice or rats, kept under controlled circadian synchronization (Lévi 1997). The latter usually consists of an alternation of 12 h of light and 12 h of darkness (Figure 1). For all these drugs, the dosing time-related difference in toxicity of the same dose usually ranges from twofold to eightfold. These rhythms in drug tolerability result from circadian changes in drug pharmacokinetics or susceptibility rhythms of target tissues. The cellular rhythms in enzymatic activities and those involved in the cell cycle regulation appear to be the main determinants of the chronopharmacology of anticancer drugs (Zhang et al 1993; Li et al 1997; Ohdo et al 1997; Tampellini et al 1998) (Table 1).

Quite strikingly, the administration of a drug at a circadian time when it is best tolerated has usually achieved the best antitumour activity. This was found for antimetabolites such as 5-fluorouracil or arabinofuranosylcytosine, for intercalating agents

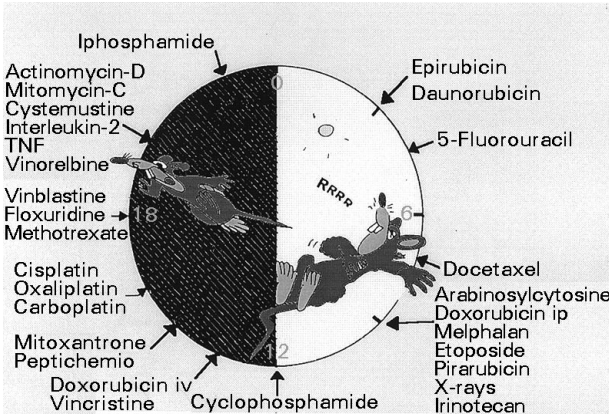

Figure 1. Circadian rhythms in anticancer drug tolerability in laboratory mice or rats. The least toxic dosing time is indicated for each cytostatic or immunologic agent as a function of the rest–activity cycle.

Table 1. Cellular determinants of circadian rhythms in tolerability for cancer chemotherapy.

Biologic function	Drug
Reduced glutathione	cisplatin, oxaliplatin
Non-protein sulphydril groups	
Enzymatic activities	
dehydropyrimidinedehydrogenase	5-fluorouracil, floxuridine
deoxythymidine kinase	
dehydrofolate reductase	methotrexate
topoisomerase I	irinotecan
0^6-alkylguanine methyltransferase	cystemustine
Cellular proliferation	
DNA synthesis (S-phase)	5-fluorouracil
	theprubicin
	irinotecan
	docetaxel
BCL-2 expression	docetaxel

such as doxorubicin, for alkylating drugs such as melphalan or cisplatin and for antimitotic drugs such as vinorelbine or docetaxel (Lévi 1997; Tampellini et al 1998). This improvement in efficacy has usually been achieved because drug doses could be safely and selectively increased by 30 to 50% at the circadian time of best tolerability.

In summary, the experimental model tells us that the circadian rhythm in drug tolerability can be used for two purposes. An improvement in quality of life can result from the dosing time-related reduction of chemotherapy toxicity, while dose and efficacy remain similar to standard schedules of delivery. An improvement in survival can result from the administration of a higher maximum tolerated dose at the least toxic circadian time as compared to other dosing times.

From mice to cancer patients: coupling of cellular rhythms to the rest–activity cycle. Cells which are engaged in DNA synthesis usually display an increased susceptibility to antimetabolites or intercalating agents. The proportions of bone marrow, gut, skin and oral mucosa cells engaged in this 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 04:00, and higher mean values between 08:00 and 20:00 (Buchi et al 1991; Smaaland et al 1991; reviewed in Lévi 1997).

Dehydropyrimidine dehydrogenase (DPD) is the rate limiting enzyme of 5-fluorouracil catabolism. Its activity in circulating mononuclear cells increases by nearly 50% between 10:00 and midnight, both in healthy subjects and in cancer patients (Harris et al 1990; reviewed in Lévi 1997).

These mechanisms of anticancer drug chronopharmacology display a similar phase relationship with the rest–activity cycle in mice and in humans, despite the former being active at night and the latter during daytime (Figure 2). For instance, 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 and near 16:00 in humans. In addition, constant rate infusion of 5-fluorouracil results in a circadian rhythm in plasma level both in mice and in cancer patients. Peak concentration in 5-fluorouracil occurs in the early rest span in both species, if the drug is infused continuously over 1 week or less.

Such apparent coupling between the circadian rest–activity cycle and several chronopharmacology mechanisms across species has been the basis for the current chronotherapy schedules which have been given to cancer patients. Multichannel programmable-in-time pumps have allowed us to test the clinical relevance of the chronotherapy principle in fully ambulatory patients, and to administer this treatment modality routinely to these patients. For so doing, it was assumed that patients exhibit similar circadian rhythms. This assumption was supported by the fact that groups of patients with metastatic or advanced breast, ovarian or colorectal cancer displayed significant circadian rhythms in blood cell counts and in plasma or serum con-

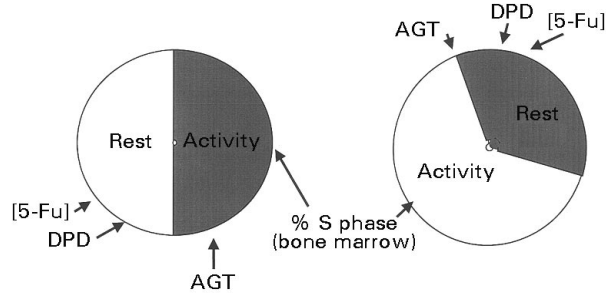

Figure 2. Coupling between chronopharmacology mechanisms and the rest–activity cycle in nocturnally-active rodents (left) and diurnally-active humans (right). DPD = dehydropyrimidine dehydrogenase, 5-Fu = 5-fluorouracil.

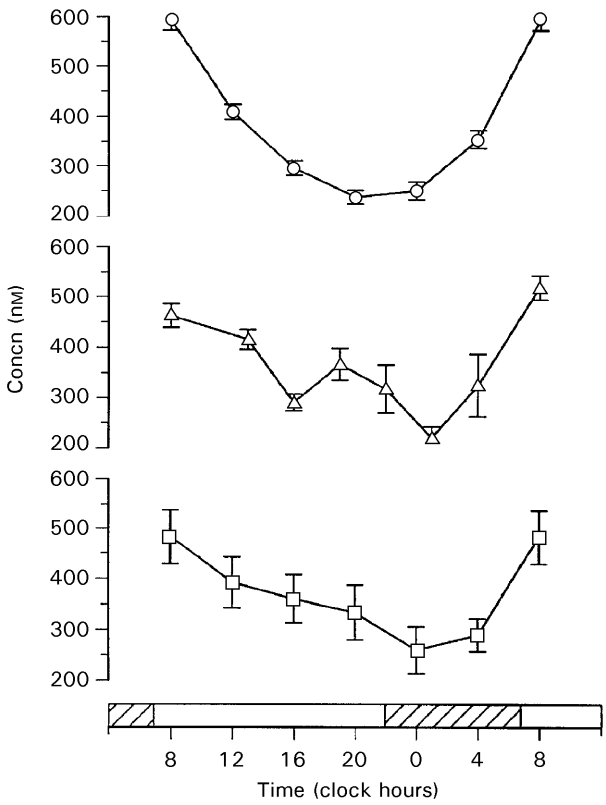

Figure 3. Total serum cortisol (mean ± s.e.m.) as a function of circadian sampling time in a group of 19 healthy subjects (○), 18 patients with metastatic colorectal cancer (△) and 20 patients with advanced ovarian cancer (□). Rhythms were statistically validated with both analysis of variance and cosinor (after Mormont et al 1998a).

centrations of cortisol, liver enzymes and creatinine (Figure 3) (Mormont & Lévi 1997). The magnitude of these average circadian changes and their relationship to the average rest–activity cycle were quite similar in cancer patients and in normal subjects.

Group chronotherapy

Two clinical trials compared the toxicity of two dosing times of anthracyclines and cisplatin in 30 patients with advanced ovarian cancer. Both studies demonstrated that doxorubicin or theprubicin were better tolerated near 6:00 and cisplatin between 16:00 and 20:00 than 12 h apart (Hrushesky 1985; Lévi et al 1990). Other limited-scale non-randomized trials were performed in patients with lung, breast or kidney cancer, with apparent improvement in therapeutic index.

Nevertheless, it became clear that the clinical relevance of the chronotherapy principle had to be tested in a large patient population using common clinical pharmacology methodology. Metastatic colorectal cancer is the second cause of cancer deaths in both men and women and its prognosis is poor with conventional treatment methods. Programmable-in-time multichannel pumps allowed the sinusoidal delivery of two or three anticancer drugs in the patient's home or during the patient's usual activities. The protocols involved the chronomodulated infusion of 5-fluorouracil and leucovorin, the reference combination chemotherapy for this disease, eventually associated with oxaliplatin, a recently discovered active drug. Maximum delivery rate of 5-fluorouracil and leucovorin was scheduled at 4:00 at night for 5-fluorouracil and leucovorin and at 16:00 for oxaliplatin, based upon an extrapolation from experimental data. Courses lasted 4 or 5 days and were repeated every 2 or 3 weeks.

The tolerability, maximum dose intensities and antitumour activity of these chronotherapy schedules were evaluated in Phase I, II and III clinical trials, involving over 1000 patients with metastatic colorectal cancer (Caussanel et al 1990; Lévi et al 1992, 1993, 1994, 1995; 1997, 1999; Bertheault-Cvitkovic 1996; Garufi et al 1997) (Table 2). Two randomized multicentre studies were performed in a total of 278 patients with this disease. All the patients received the three-drug regimen either as a constant rate infusion or as a chronomodulated administration. Chronotherapy reduced the incidence of severe mucositis by a factor of five, halved

Table 2. Clinical trials of chronotherapy in 1275 patients with metastatic colorectal cancer.

Phase of study	Drug treatment	Schedule	Number of patients	Reference
I	5-Fluorouracil	5 days every three weeks	35	Lévi et al 1995
	5-Fluorouracil + leucovorin	5 days every three weeks	34	Garufi et al 1997
	Oxaliplatin	5 days every three weeks	25	Caussanel et al 1990
	5-Fluorouracil + leucovorin + oxaliplatin	4 days every two weeks	114	Lévi et al (unpublished)
II multicentre	5-Fluorouracil + leucovorin	5 days every three weeks	43	Chollet et al 1994
II multicentre	5-Fluorouracil + leucovorin	4 days every two weeks	100	Curé et al 1998
II multicentre	5-Fluorouracil + leucovorin	14 days every four weeks	67	Bjarnason et al 1998
II multicentre	Oxaliplatin	5 days every three weeks	30	Lévi et al 1993
	5-Fluorouracil + leucovorin + oxaliplatin	5 days every three weeks	93	Lévi et al 1992
	5-Fluorouracil + leucovorin + oxaliplatin	4 days every two weeks	54	Brienza et al 1993
II multicentre	5-Fluorouracil + leucovorin + oxaliplatin	4 days every two weeks	50	Bertheault-Cvitkovic et al 1996
II multicentre	5-Fluorouracil + leucovorin + oxaliplatin	4 days every two weeks	62	Lévi et al unpublished
II multicentre	5-Fluorouracil + leucovorin + oxaliplatin	4 days every two weeks	90	Lévi et al 1999
III multicentre	5-Fluorouracil + leucovorin + oxaliplatin flat vs chrono	4 days every two weeks	92	Lévi et al 1994
III multicentre	5-Fluorouracil + leucovorin + oxaliplatin flat vs chrono	5 days every three weeks	186	Lévi et al 1997
III multicentre	Chrono 5-Fluorouracil + leucovorin with or without oxaliplatin	5 days every three weeks	200	Giacchetti et al 1997

Table 3. Main results from a randomized multicenter trial comparing flat vs chronomodulated chemotherapy in 186 patients with metastatic colorectal cancer.

Effect	Percentage of patients		*P*
	Flat	Chrono	
Hospitalization for toxicity	31	10	0·001
Severe mucositis	76	14	0·0001
Functional impairment (peripheral sensory neuropathy)	31	16	0·01
Tumour response > 50%	29	51	0·003

Data are from Lévi et al 1997.

that of functional impairment from peripheral sensory neuropathy and reduced by a factor of three the incidence of grade 4 toxicity requiring hospitalization, as compared with the flat infusion regimen. This improvement in tolerability was accompanied by a significant increase in objective response rate from 29 to 51% (Table 3, Lévi et al 1994, 1997). Further dose escalation of chronotherapy was possible and achieved 66% of objective tumour responses in a multicentre setting (Bertheault-Cvitkovic et al 1996; Lévi et al 1999). This antitumour efficacy was 3 to 4-fold greater than that achieved with conventional regimens involving 5-fluorouracil and leucovorin. The results clearly emphasise that group chronotherapy improves the therapeutic index of the chemotherapy of colorectal cancer.

Nevertheless one can wonder whether the magnitude of improvement brought about by such fixed chronotherapy schedule varies according to individual circadian system function. Thus altered circadian rhythms were found in tumour-bearing animals and in cancer patients.

Circadian System Alterations During Cancer Processes

Experimental data

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 tumour 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 tumours, 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 tumours. The presence of a cancer also altered the normal rhythms in plasma corticosterone, body temperature or bone marrow DNA synthesis in some rodent tumour models, but not in others (reviewed in Mormont & Lévi 1997).

Rhythms in human tumours

As early as 1953, daily variations in the mitotic index of human mammary carcinoma and squamous or basal cell carcinoma were described, with interindividual variations (Voutilainen 1953; Tahti 1956). Re-analysis of these data validated a circadian rhythm in a group of six women with breast cancer, with a maximum near 15:00 h. Ultradian rhythms were found in the group of 31 patients with squamous or basal cell carcinoma (Garcia-Sainz & Halberg 1966). Progressive dampening of skin mitotic activity was also suggested in patients with actinic keratoses or skin cancer (Zagula-Mally et al 1979).

More recently, cell cycle related parameters of tumour cells and normal mesothelial cells were studied around the clock within the peritoneal lavage fluid from 30 patients with ovarian cancer (Klevecz et al 1987). A circadian maximum in DNA synthesis of both diploid and aneuploid tumour cells was found between 12:00 and 16:00 h. This time was almost 12 h out of phase with the peak of DNA synthesis in mesothelial cells. Ultradian rhythms, with 8-h and 12-h periods, were also documented in the aneuploid tumour cell population (Klevecz & Braly 1991).

Twenty four-hour changes were described for DNA synthesis in malignant lymph nodes from 24 patients with non-Hodgkin's lymphoma. The maximum occurred near midnight, while the peak of the S phase was usually found between 12:00 h and 16:00 h in the bone marrow of healthy subjects (Smaaland et al 1993). Interestingly, when patients were classified according to tumour stage, a circadian rhythm in DNA synthesis was validated in the group of patients with an early-stage tumour, but not in the group of patients with stage-IV lymphoma. This observation suggested a link between cancer stage and circadian rhythm alteration.

Noteworthy observations on breast skin temperature indicated that circadian rhythms persisted on the surface of breast cancers that were slow-growing and well-differentiated. Rhythms were however dampened, with maxima occurring 6 h earlier than in the non-cancerous breast. Con-

Table 4. Rhythms in human tumours.

Cancer type	Variable	Circadian rhythm	Peak different from normal tissue
Breast	Surface temperature	Yes	Yes
	^{32}P uptake	Yes	Yes
	Mitotic index	Yes	Not specified
Cervix	Surface temperature	Yes	Yes
Ovary	Cell cycle distribution	Yes[a]	Yes
Non-Hodgkin's lymphoma	Cell cycle distribution	Yes[b]	Yes

[a]Variable according to ploidy, [b]variable according to stage. After Mormont & Lévi (1997).

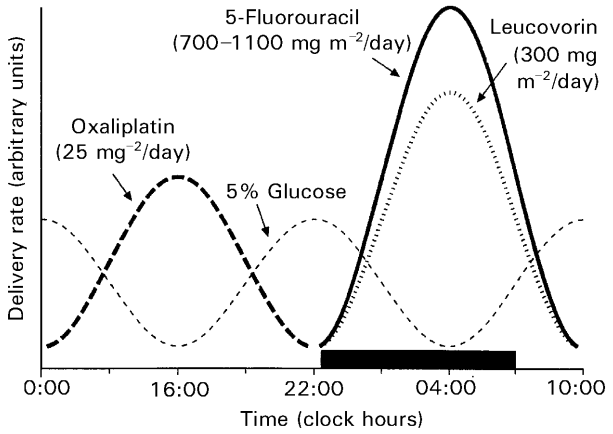

Figure 4. Example of a chronomodulated three-drug delivery scheme, used against colorectal cancer metastases. All three drugs (5-fluorouracil, leucovorin, and oxaliplatin) are administered automatically for 4 to 5 days, using a programmable multichannel pump, which does not require patient hospitalization.

versely, fast growing, poorly differentiated tumours displayed a shortening in the period of tumour temperature rhythm (Gautherie & Gros 1977). Another study, on 14 patients, had also shown evidence of a phase advance of approximately 6 h, in the rhythm of skin temperature of the cancerous breast, as compared with the contralateral breast (Mansfield et al 1973). Table 4 summarizes the main results published on human tumour circadian and ultradian rhythms.

Individual rhythms in cancer patients

Despite the mean proportion of bone marrow cells in the S-phase being significantly greater at noon than at midnight in eleven patients with advanced cancer, no time-related difference was apparent in four of these patients, whose cortisol rhythm was suppressed or inverted (Smaaland et al 1992). Studies on cortisol and other blood parameters associated with circadian rhythms were performed in 51 patients with advanced or metastatic ovarian, breast or colorectal cancer, with a minimum of 10 blood samples to estimate individual rhythmicity (Touitou et al 1995, 1996; Mormont et al 1998a, b). Once more a circadian rhythm was statistic-ally validated for each group of patients (Figure 4). Nevertheless, the 24-h rhythms in plasma cortisol and other variables were prominent in some patients and apparently suppressed in others, despite the lack of any glucocorticoid medication. These rhythm alterations were mostly found in patients with poor performance status (graded as 2 to 4, according to the WHO scale) or large tumour burden.

A large study was undertaken in 200 patients with metastatic colorectal cancer to estimate the incidence of circadian system alterations, as assessed from rest–activity and cortisol rhythms, in a population of patients eligible for clinical trials, i.e. with a performance status ≤ 2. This circadian system assessment was as little invasive as possible, and did not require hospitalization. Motor activity was continuously monitored for 3 days, using a wrist actigraphy bracelet, and a blood sample was obtained at 8:00 and at 16:00 on two consecutive days in each patient. The rest–activity cycle can be appropriately measured with a 3-day recording. The strength of the circadian component was assessed with an autocorrelation coefficient at 24 h (r24). 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 a low normal limit (Mormont et al 1998a). Thirty percent of the 200 patients had an abnormal cortisol rhythm using this criterion (Mormont et al 1998b). 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 (Mormont 1998). Nevertheless, only a weak correlation was found between cortisol rhythm and rest–activity cycle alterations. This suggests that both rhythms are controlled by different circadian oscillators or circadian clock pathways.

Clinical relevance of individual circadian system function

The status of the circadian system was first tested as an estimate of a cancer patient's prognosis in two pilot studies respectively involving 20 patients

with advanced ovarian cancer and 13 patients with metastatic breast cancer. Significant correlations were found between individual circadian amplitude of plasma cortisol or circulating leucocyte count and well-known prognostic factors of response and survival (Benavides 1991).

The above-mentioned study prospectively investigated the relevance of circadian system function for quality of life and survival in 200 patients with metastatic colorectal cancer. Results have indicated that the circadian distribution of activity was well correlated to several quality of life parameters from the EORTC QLQ-30 questionnaire and constituted a joint prognostic factor of survival, independently from the well-known prognostic factors in this disease (Mormont et al 1997; Mormont et al 1998b).

The results suggest that circadian system function may play an important role for cancer patient outcome, an issue which deserves further investigation. Thus, specific treatment of circadian dysfunctions may help improve the status and outcome of cancer patients, and contribute in enhancing the therapeutic efficacy of chrono-modulated chemotherapy. The clinical relevance of chronotherapy for the outcome of cancer patients is being investigated along these lines within the E.O.R.T.C. for colon, pancreas and breast carcinoma.

References

Benavides, M. (1991) Cancer Avance de l'Ovaire: Approche Chronobiologique comme Nouvelle Strategie du Traitement et de la Surveillance Clinique et Biologique. PhD Thesis, Universite Paris XI

Bertheault-Cvitkovic, F., Jami, A., Ithzaki, M., Brummer, P. D., Brienza, S., Adam, R., Kunstlinger, F., Bismuth, H., Misset, J. L., Lévi, F. (1996) Biweekly intensified ambulatory chronomodulated chemotherapy with oxaliplatin, 5-fluorouracil and folinic acid in patients with metastatic colorectal cancer. J. Clin. Oncol. 14: 2950–2958

Bjarnason, G. A., Marsh, R., Chu, N. M., Kerr, I., Franssen, E. (1998) Phase II study of 5-fluorouracil (FU) and leucovorin LV) by a 14 day chronomodulated infusion in patients with metastatic colorectal cancer. Proc. Am. Soc. Clin. Oncol. 17: 275a

Brienza, S., Lévi, F., Valori, V. (1993) Intensified (every two weeks) chronotherapy with 5-fluorouracil (5-FU), folinic acid (FA) and oxaliplatin (L-OHP) in previously treated patients with metastatic colorectal cancer. Proc. Am. Soc. Clin. Oncol. 12: 197

Buchi, K. N., Moore, J. G., Hrushesky, W. J., Sothern, R. B., Rubin, N. H. (1991) Circadian rhythm of cellular proliferation in the human rectal mucosa. Gastroenterology 101: 410–415

Caussanel, J. P., Lévi, F., Brienza, S., Misset, J. L., Itzhaki, M., Adam, R., Milano, G., Hecquet, B., Mathe, G. (1990) Phase I trial of 5-day continuous venous infusion of oxaliplatin at circadian rhythm-modulated rate compared with constant rate. J. Natl Cancer Inst. 82: 1046–1050

Chollet, P., Curé, H., Garufi, C. (1994) Phase II trial with chronomodulatd 5-fluorouracil (5-FU) and folinic acid (FA) in metastatic colorectal cancer. Proc. 6th Intern. Conf. Chronopharm. Chronother., Amelia Island FL, July 5–9, Abstract VIIIb-4

Curé, H., Adenis, A., Tubiana-Mathieu, N., Ouabdesselam, R., Kwiatkowski, F., Perpoint, B., Coudert, B., Focan, C., Giachetti, S., Lévi, F., Chollet, P. (1998) Phase II trial of chronomodulated (CM) high dose 5-fluorouracil (5-FU) and l-folinic acid (l-FA) in patients with metastatic colorectal cancer (MCC). Proc. Am. Soc. Clin. Oncol. 17: 273a

Depres-Brummer, P., Metzger, G., Lévi, F. (1998) Pharmacologic restoration of suppressed temperature rhythms in rats with melatonin, melatonin receptor agonist S20242 or 8-hydroxy-2-(di- *n*-propylamino)tetralin (8-OH-DPAT). Eur. J. Pharmacol. 347: 57–66

Garcia-Sainz, M., Halberg, F. (1966) Mitotic rhythms in human cancer reevaluated by electronic computer programs. Evidence for chronopathology. J. Natl Cancer Inst. 37: 279–292

Garufi, C., Lévi, F., Aschelter, A. M., Pace, R., Giunta, S., Nistico, C., Galla, D. A., Silecchia, G. F., Franchi, F., Narduzzi, C., Terzoli, E. (1997) A phase I trial of five day chronomodulated infusion of 5-fluorouracil and 1-folinic acid in patients with metastatic colorectal cancer. Eur. J. Cancer 33: 1566–1571

Gautherie, M., Gros, C. (1977) Circadian rhythm alteration of skin temperature in breast cancer. Chronobiologia 4: 1–17

Green, C. (1998) How cells tell time. Trends Cell Biol. 8: 224–230

Harris, B., Song, R., Soong, S., Diasio, R. B. (1990) Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels : evidence for circadian variation of plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res. 50: 197–201

Hrushesky, W. (1985) Circadian timing of cancer chemotherapy. Science 228: 73–75

Klein, D. C., Moore, R. Y., Reppert, S. M. (1991) Suprachiasmatic Nucleus, the Mind's Clock. Oxford University Press, Oxford

Klevecz, R., Braly, P. (1991) Circadian and ultradian cytokinetic rhythms of spontaneous human cancer. Ann. N.Y. Acad. Sci. 618: 257–276

Klevecz, R., Shymko, R., Braly, P. (1987) Circadian gating of S phase in human ovarian cancer. Cancer Res. 47: 6267–6271

Lévi, F. (1997) Chronopharmacology of anticancer agents. In: Redfern, P. H., Lemmer, B. (eds) Physiology and Pharmacology of Biological Rhythms. Handbook of Experimental Pharmacology. Vol. 125, Springer-Verlag, Berlin, pp 299–331

Lévi, F., Benavides, M., Chevelle, C., Le Saunier, F., Bailleul, F., Misset, J. L., Regensberg, C., Vannetzel, J. M., Reinberg, A., Mathe, G. (1990) Chemotherapy of advanced ovarian cancer with 4′-*O*-tetrahydropyranyl adriamycin (THP) and cisplatin: a phase II trial with an evaluation of circadian timing and dose intensity. J. Clin. Oncol. 8: 705–714

Lévi, F., Misset, J. L., Brienza, S., Adam, R., Metzger, G., Itzakhi, M., Caussanel, J. P., Kunstlinger, F., Lecouturier, S., Descorps-Declere, A. (1992) A chronopharmacologic phase II clinical trial with 5-fluorouracil, folinic acid and oxaliplatin using an ambulatory multichannel programmable pump. High antitumor effectiveness against metastatic colorectal cancer. Cancer 69: 893–900

Lévi, F., Perpoint, B., Garufi, C., Focan, C., Chollet, P., Depres-Brummer, P., Zidani, R., Brienza, S., Itzhaki, M., Iacobelli, S. (1993) Oxaliplatin activity against metastatic colorectal cancer. A phase II study of 5-day continuous venous infusion at circadian rhythm modulated rate. Eur. J. Cancer 29A: 1280–1284

Lévi, F. A., Zidani, R., Vannetzel, J. M., Perpoint, B., Focan, C., Faggiuolo, R., Chollet, P., Garufi, C., Itzhaki, M., Dogliotti, L. (1994) Chronomodulated versus fixed infusion rate delivery of ambulatory chemotherapy with oxaliplatin, 5-fluorouracil and folinic acid in patients with colorectal cancer metastases. A randomized multiinstitutional trial. J. Natl Cancer Inst. 86: 1608–1617

Lévi, F., Soussan, A., Adam, R., Caussanel, J. P., Metzger, G., Jasmin, C., Bismuth, H., Smolensky, M., Misset, J. L. (1995) A phase I-II trial of five-day continuous intravenous infusion of 5-fluorouracil delivered at circadian rhythm modulated rate in patients with metastatic colorectal cancer. J. Infus. Chemother. 5: 153–158

Lévi, F., Zidani, R., Brienza, S., Dogliotti, L., Perpoint, B., Rotarski, M., Letourneau, Y., Llory, J. F., Chollet, P., Le Rol, A., Kanoui, A., Focan, C., Giachetti, S., Bertheault-Cvitkovic, F., Kunstlinger, F., Adam, R., Itzhaki, M., Jasmin, C., Misset, J. L. (1999) A multicenter evaluation of intensified ambulatory chronomodulated chemotherapy with oxaliplatin, fluorouracil and leucovorin as initial treatment of patients with metastatic colorectal cancer. Cancer 85: 2532–2540

Lévi, F., Zidani, R., Misset, J. L. (1997) Randomized multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. Lancet 350: 681–686

Li, X. M., Metzger, G., Filipski, E., Boughattas, N., Lemaigre, G., Hecquet, B., Filipski, J., Lévi, F. (1997) Pharmacologic modulation of reduced glutathione (GSH) circadian rhythms by buthionine sulfoximine (BSO): relationship with cisplatin (CDDP) toxicity in mice. Toxicol. Appl. Pharmacol. 143: 281–290

Mansfield, C. M., Carabasi, R. A., Wells, W., Borman, K. (1973) Circadian rhythm in the skin temperature of normal and cancerous breasts. Intl J. Chronobiol. 1: 235–243

Mormont, C. (1998) Selection et Validation d'un Test Predictif de la Survie des Patients Atteints de Cancer Colorectal Metastase, Fonde sur l'Alteration du Systeme Circadien. PhD Thesis, Universite Paris XI

Mormont, M. C., Lévi, F. (1997) Circadian-system alterations during cancer processes: a review. Int. J. Cancer 70: 241–247

Mormont, M. C., Bleuzen, P., Lellouch, J. (1997) Prognostic value of circadian rhythm assessment for survival of patients with metastatic colorectal cancer. Proc. 33rd Ann. Meeting Am. Soc. Clin. Oncol., May 17–20,1997, Denver, CO, U.S.A., Abstract number 956

Mormont, M. C., Giacchetti, S., Waterhouse, J. (1998a) Activity circadian rhythm as an independent prognostic factor of survival in patients (pts) with metastatic colorectal cancer (MCC). Proc. 34th Ann. Meeting Am. Soc. Clin. Oncol. Abstract number 1032

Mormont, M. C., Hecquet, B., Bogdan, A., Benavides, M., Touitou, Y., Lévi, F. (1998b) Non-invasive estimation of the circadian rhythm in serum cortisol in patients with ovarian or colorectal cancer. Int. J. Cancer 78: 421–424

Ohdo, S., Makinosumi, T., Ishizaki, T., Yukawa, E., Higuchi, S., Nakano, S., Ogawa, N. (1997) Cell cycle-dependent chronotoxicity of irinotecan hydrochloride in mice. J. Pharmacol. Exp. Ther. 283: 1383–1388

Plautz, J. D., Kaneko, M., Hall, J. C., Kay, S. A. (1997) Independent photoreceptive circadian clocks throughout Drosophila. Science 278: 1632–1635

Smaaland, R., Abrahamsen, J. F., Svardal, A. M., Lote, K., Ueland, P. M. (1992) DNA cell cycle distribution and glutathione (GSH) content according to circadian stage in bone marrow of cancer patients. Br. J. Cancer 66: 39–45

Smaaland, R., Lote, K., Sothern, R. B., Laerum, O. D. (1993) DNA synthesis and ploidy in non-Hodgkin's lymphomas demonstrate variation depending on circadian stage of cell sampling. Cancer Res. 53: 3129–3138

Smaaland, R., Laerum, O. D., Lote, K., Sletvold, O., Sothern, R. B., Bjerknes, R. (1991) DNA synthesis in human bone marrow is circadian stage dependent. Blood 77: 2603–2611

Tahti, E. (1956) Studies of the effect of X-irradiation on 24 hour variations in the mitotic activity in human malignant tumors. Acta Path. Microbiol. Scand. 117: 1–61

Tampellini, M., Filipski, E., Liu, X. H., Lemaigre, G., Li, X. M., Vrignaud, P., Francois, E., Bissery, M. C., Lévi, F. (1998) Docetaxel chronopharmacology in mice. Cancer Res. 58: 3896–3904

Tei, H., Okamura, H., Shigeyoshi, Y., Fukuhara, C., Ozawa, R., Hirose, M., Sakaki, Y. (1997) Circadian oscillation of a mammalian homologue of the Drosophila period gene. Nature 89: 512–516

Touitou, Y., Haus, E. (1992) Biological Rhythms in Clinical and Laboratory Medicine, Springer-Verlag, Berlin

Touitou, Y., Lévi, F., Bogdan, A., Benavides, M., Bailleul, F., Misset, J. L. (1995) Circadian desynchronization of blood variables in patients with metastatic breast cancer. Role of prognostic factors. J. Cancer Res. Clin. Oncol. 121:181–188

Touitou, Y., Bogdan, A., Lévi, F., Benavides, M., Auzeby, A. (1996) Disruption of the circadian patterns of serum cortisol in breast and ovarian cancer patients. Relationships with tumor marker antigens. Br. J. Cancer 74: 1248–1252

Vitaterna, M. H., King, D. P., Chang, A. M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., Dove, W. F., Pinto, L. H., Turek, F. W., Takahashi, J. S. (1994) Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior. Science 264: 719–725

Voutilainen, A. (1953) Uber die 24-stunden-rhythmik der mitozfrequenz in malignen Tumoren. Acta Path. Microbiol. Scand. 99 (Suppl.): 1–104

Zagula-Mally, Z. W., Cardoso, S. S., Williams, D. (1979) Time point differences in skin mitotic activity of actinic keratoses and skin cancers. In: Rainberg, A., Halberg, F. (eds) Chronopharmacology, Pergamon Press, New York, pp 399–402

Zhang, R., Lu, Z., Liu, T., Soong, S. J., Diasio, R. B. (1993) Relationship between circadian-dependent toxicity of 5-fluorodeoxyuridine and circadian rhythms of pyrimidine enzymes: possible relevance to fluoropyrimidine chemotherapy. Cancer Res. 53: 2816–2822