The circadian timing system, a coordinator of life processes. Implications for the rhythmic delivery of cancer therapeutics

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Abstract - **Cell physiology is regulated along the 24-h time scale by a circadian timing system composed of molecular clocks within each cell and a central coordination system in the brain. The mammalian molecular clock is made of interconnected molecular loops involving at least 12 circadian genes. The cellular clocks are coordinated by the suprachiasmatic nucleus, a hypothalamic pacemaker which also helps the organism adjust to environmental cycles. The rest-activity rhythm is a reliable marker of the circadian system function in both rodents and Man. It can be monitored non-invasively through several devices or systems. The circadian organization is responsible for predictable changes in the tolerability and efficacy of anticancer agents, and also controls tumor promotion and growth. The clinical relevance of the chronotherapy principle, i.e. treatment administration as a function of rhythms, has been demonstrated in randomized multicenter trials, using programmable-in-time drug delivery systems. Chronotherapeutic schedules first documented the safety and the activity of oxaliplatin-based combination chemotherapy in patients with metastatic colorectal cancer. The chronotherapy concept offers further promises for improving current cancer treatment options as well as for optimizing the development of new anticancer or supportive agents. Technological developments of chronotherapeutics in daily practice are essential in order to non invasively assess dynamic changes in biological functions and to insure temporally-adjusted therapeutics interventions.**

I - The circadian timing system

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

Circadian rhythms are genetically fixed. For instance, mutations of the circadian genes *per* in Drosophila, in mouse or in humans result in severe disturbances of the rest-activity circadian cycle, which translate into modifications of the period, amplitude or acrophase pending upon experimental conditions (1-3).

The light perceived by the visual pathways and the secretion of melatonin, a hormone released by the pineal gland during darkness, help to reset the internal clock that regulates the timing of different body functions. A hypothalamic structure, the suprachiasmatic nucleus (SCN), plays a key role in the coordination of circadian rhythms (1, 4).

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

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

Locomotor activity reliably reflects circadian clock function in several animal species. Its endogenicity was demonstrated by its persistence in constant environmental conditions in flies, rodents and humans. This rhythm is controlled by the molecular clock genes in mammals. Direct pharmacologic actions targeted at the SCN in rodents translate into a phase shift of the rest/activity rhythm of the animals. In rodents, the physical destruction of the SCN results in a complete suppression of the rest/activity rhythm, while the transplantation of SCN restores circadian rhythmicity. These experimental facts clearly demonstrate the dependency of this rhythm upon SCN function (1, 5).

In man, the rest-activity rhythm is considered and used as a marker of the circadian timing system in isolation studies, in phase shift studies, and in psychiatry. The rest-activity rhythm can be easily measured using a small-size instrument worn on the wrist, and called an actigraph. As wrist monitoring of activity is totally non-invasive, there is no restriction to its use in cancer patients, even in an ambulatory

setting (6). The easy recording of rest-activity has further supported its use as a reference rhythm for the circadian timing of medications and for the evaluation of circadian clock function.

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

The complex machinery of the molecular clock (1, 3) was recently shown to exert a negative control on the transcriptional activity of some key genes involved into cell cycle regulation, thereby suggesting that the circadian clock could regulate cell proliferation. Circadian rhythms have been extensively reported for cell cycle phase distribution in healthy or malignant mammalian tissues (7-12). Two recent studies have further identified *c-myc*, *p53* and *wee1* as being clock-controlled genes (10-13). *C-myc* and *wee1* respectively promote cell cycle progression from G1 to S and from G2 to M. Furthermore, *c-myc* can also exert proapoptotic effects through p53-dependent or independent pathways.

Many other genes that control cell cycle progression or apoptosis (10-13) display 24-h rhythms in mRNA and/or protein expression in healthy tissues from rodents and/or humans, also equipped with molecular clock components.

IV - Experimental chronopharmacology of anticancer drug

Circadian dosing time influences the extent of toxicity of ~30 anticancer drugs, including cytostatics and cytokines, in mice or rats. For all these drugs, survival rate vary by 50% or more according to circadian dosing time of a potentially lethal dose. Mechanisms involve dosing-time dependencies in drug pharmacokinetics and pharmacodynamics. They result from the circadian control of drug metabolism, cellular detoxification and proliferation and DNA repair (4, 14).

Quite strikingly, the administration of a drug at a circadian time when it is best tolerated has usually achieved best antitumor activity (9, 14, 15). The reproducible coincidence between times of highest efficacy and least toxicity for most anticancer agents suggest that common mechanisms are involved.

V - Clinical chronopharmacology and cancer chronotherapeutics

Short intravenous infusions of several anticancer drugs such as cisplatin, doxorubicin or 5-fluorouracil (5-FU), or oral intake of busulfan were associated with modifications of plasma and/or urinary pharmacokinetics according to dosing time. Continuous intravenous infusion of 5-FU, doxorubicin or vindesine also resulted in circadian changes in plasma drug levels, despite a flat infusion rate (16). Interpatient variability in circadian time-dependent pharmacokinetics were also observed.

The activity of dihydropyrimidine dehydrogenase (DPD), the initial enzyme for the catabolism of 5-FU, was studied around the clock in peripheral blood mononuclear cells of patients suffering from a gastrointestinal tumor, with higher DPD activity at early night, near midnight or 4:00 h (13, 16).

Cell proliferation is also likely to be one mechanism involved, as cells which are engaged into DNA synthesis usually display an increased susceptibility to antimetabolites or intercalating agents. The proportion of bone marrow, gut, skin and oral mucosa cells engaged in the S-phase of the cell division cycle vary by 50% or more along the 24-h time scale in healthy human subjects. For all these tissues, lower mean values occur between midnight and 4:00 during the night, and higher mean values between 08:00 and 20:00 (7, 8).

These mechanisms of anticancer drug chronopharmacology display a similar phase relationship with the rest-activity cycle in mice and in humans, despite the fact that the former are active at night and the latter during daytime. Similarly, DPD activity peaks during early light in mice or rats and at early night in humans. For instance, 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-FU occurs in the early rest span in both species, if the drug is infused continuously over 1 week or less (14, 16).

The apparent coupling between the circadian rest-activity cycle and several chronopharmacology mechanisms across species has been the basis for the chronotherapy schedules which have been given to cancer patients. As a working hypothesis, expected times of least toxicity in human patients were extrapolated from those experimentally demonstrated in mice or rats, by referring them to the respective rest/activity cycle of each species, e.g. with ~12 h time lag. For instance, least toxicity of 5-FU occurred near 5 Hours After Light Onset in mice and was predicted to correspond to 04:00 h in human subjects, resting from 23:00 h to 07:00 h (17).

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

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

Chronomodulated chemotherapy using programmable pumps

The clinical relevance of the chronotherapy principle was mainly tested in a large population of patients with metastatic colorectal cancer, using the standard methodology of clinical trials (14, 18). Metastatic colorectal cancer is the second most common cause of cancer deaths in both genders, and its conventional treatment methods did not offer many therapeutic possibilities other than the reference combination chemotherapy of 5-FU and leucovorin (LV) until the mid-nineties. The chronomodulated protocols involved the time-qualified infusion of 5-FU-LV, eventually associated with oxaliplatin (l-OHP), an active drug more recently recognized. Maximum delivery rate of 5-FU-LV was scheduled at 04:00 at night, and of l-OHP at 16:00 (chronoFLO). Courses lasted 4 or 5 days and were repeated every 2 or 3 weeks.

The tolerability, maximum dose intensities and antitumor activity of these chronotherapy schedules were evaluated in Phase I, II and III clinical trials, involving over 2000 patients with metastatic colorectal cancer. In two consecutive multicenter trials, chronoFLO was compared to constant rate infusion of the same drugs. ChronoFLO reduced the incidence of severe mucositis five-fold, halved that of functional impairment from peripheral sensory neuropathy and reduced threefold the incidence of grade 4 toxicity requiring hospitalization, as compared to the flat infusion regimen. This improvement in tolerability was accompanied with a significant increase in antitumor activity (objective response rate) from 29% to 51% (4, 18). The good tolerability of chronotherapy further allowed its dose-intensification by administering a 4-day cycle every 2weeks, and by increasing the dose of 5-FU. A further randomized trial has been undertaken in 564 patients with metastatic colorectal cancer by the Chronotherapy Group of the European Organization for Research and Treatment of Cancer (EORTC). This study compared the two most active schedules administering 5-FU, LV and l-OHP near maximum tolerated dose : chronomodulated infusion of the 3 drugs over 4 days (so called, chrono FLO4) vs 44-h infusion of 5-FU on days 1 and 2 and 2-h infusions of oxaliplatin and leucovorin on day 1 and leucovorin on day 2 (FOLFOX2). A non significant trend toward a better survival was found in the patients receiving chronoFLO4. The study further revealed a major role of gender as a predictor of optimal schedule. Thus chronoFLO4 was of no benefit in women. However a significant improvement in median survival was achieved in men receiving chronotherapy, as compared to the men given conventional regimen (19).

VI - Discussion and perspectives

Malignant tumors and cancer-bearing hosts may exhibit nearly normal or markedly altered circadian rhythms. Rhythm alterations seem to depend upon tumor type, growth rate and level of differentiation, both in animal and human tumors, and they usually worsen along the course of cancer progression (9, 13-16).

Moreover, the rest-activity rhythm is a positive prognostic factor of both tumor response and survival in patients with metastatic colorectal cancer (6, 14). The rest/activity rhythm also seems to be a biological determinant of the welfare of cancer patients. These results open novel perspectives towards understanding the impact of cancer-induced circadian system alterations on host physical and psychological balance.

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

Most likely marked benefit from chronotherapeutics will stem from the tailoring of rhythmic delivery to the individual features of the circadian timing system through novel technological developments.

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