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# Circadian cancer pharmacodynamics

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**Summary.** - After a large recognition of experiments and concepts developed in the centuries on the pharmacological treatment of human diseases, the article concisely provides the state of the art on chronotherapy of cancer. The techniques and results are illustrated. The perspectives of optimizing cancer therapy via optimal circadian schemes of pharmacological agents are clearly suggested.

*Key words*: cancer, chronobiology, chronopharmacology, circadian rhythms, chronotherapy.

**Riassunto** *(Farmacodinamica circadiana del cancro).* - Dopo una ampia ricognizione degli esperimenti e dei concetti sviluppatisi nei secoli sul trattamento farmacologico delle malattie umane, l'articolo concisamente fa il punto della situazione sulla cronoterapia delle neoplasie maligne. Vengono illustrati tecniche e risultati. Si lasciano chiaramente intravedere le prospettive di ottimizzazione della terapia del cancro attraverso gli schemi modulati sul tempo circadiano della somministrazione degli agenti antineoplastici.

*Parole chiave*: cancro, cronobiologia, cronofarmacologia, cronoterapia, ritmi circadiani.

## Introduction

Revolutions have occurred periodically for millennia across the cultural faces of medicine. Some have focused upon a single therapy, have perfected and applied it more and more broadly until its use attained ceremonial and even religious significance. Others have provided broad frameworks from which physiology and the balance between health and disease could be better understood.

The recognition of contagion, the generalization of evolution and ideas of natural selection, and the understanding of heritability, each have had revolutionary biomedical consequences. Each of these revolutionary concepts have had more than a century to impact medicine.

The early 19th century epidemiologic recognition of contagion through isolation of the Broad Street pump as the point source for an epidemic of cholera closely followed by the experiment of Semmelweis proving that puerperal sepsis was transmitted from the autopsy room to the labor and delivery suite (on the unwashed hands of medical students); each led to Koch's development of microbiology. The full range of therapies for microbiologic diseases from Ehrlich's Salversan through Domagk's sulfa and Fleming's penicillin as well as the subsequent understanding of immune networks flow from the Broad Street pump and the Vienna obstetric wards.

The impact of the generalization of the evolutionary concept of natural selection in biomedicine has included: the recognition of the tendency of selective pressures to mold patterns of subcellular and cellular function, to organize tissue and organ function and to integrate individuals, societies and ecosystems. Temperospatial gradients responsible for embryologic development, cellular and tissue differentiation; the establishment, erasure and renewal of specific immune network response; the continuous interactive renewal, updating and remodeling of neurologic structure and function associated with learning, forgetting and relearning; as well as the selection of bacterial or cellular drug resistance; or the development, emergence as well as the predominance of effectively metastatic clones.

The development of an appreciation for, a deepening understanding of and most recently the technical mastery, over heritability is leading medicine into the next millennium. This revolution beginning with Mendel's observations accelerated by Haldane's elegant descriptions and predictions, broadened by McClintock's experimentation and given substance by Watson and Crick now serves as the basis for a pharmacopeia of unimagined power and specificity.

An incipient revolution as pervasive as the three delineated above has been germinating for half a century. The footprints of the founders of this revolution are still fresh. Most of its progenitors are still productive scientists. Aschoff, Halberg, Pittendrigh and Hastings are the living pioneers of the discipline of chronobiology who with Mills, Bunning, Brown and Richter, among others, hammered out discoveries of the endogenicity of circadian time structure; observed the behavior of that time structure in various control and experimental situations; discovered and quantified biological rhythms of other frequency ranges; and determined their ubiquity to include *homo sapiens*. Members of the second generation of chronobiologist investigators are just now hitting their stride in terms of maximum scientific productivity and leadership while the third generation of biodynamicists has just been initiated.

This quantitative biodynamic revolution has awaited the technical capability to measure precisely and often; to effectively handle long series of dense biodynamic data and to complexly model multiple interacting rhythmic variables. Further developments in this revolution stand in the path of effective applications of molecular genetic advances relevant for disease prevention, earlier diagnosis and better disease treatment. The application of all therapies with less than perfect toxic/therapeutic ratios requires a quantitative consideration of treatment timing across several relevant frequency ranges.

Investigation of the mechanisms by which cells and organisms order tasks in time have benefited immensely by the growing understanding of genetics. Mutants with heritably abnormal time structure have been discovered, isolated and bred. Single gene mutations predictably affecting time keeping have been defined and «cellular time keeping genes» cloned from a variety of species their striking homology noted and their gene products are currently being defined. The mechanisms and hierarchy of organismic temporal coordination have also recently been more clearly defined using neural transplantation techniques in drosophila and hamster. These studies have defined the primary, site of organismic ultradian and circadian temporal coordination and shown its transplantation to transfer the donor's time structure to the recipient organism.

Time structure has evolved to maximize biological economy and to ensure systematic stability. Biological economy demands that subcellular, cellular, tissue, organ, organismic, societal and ecosystem tasks be initiated and completed at specific optimal times within important time-keeping cycles and in a certain order relative to one another. The absolute need for systematic stability requires continuous readjustment of the system to external and internal dynamic requirements. This may be pictured as a cyclical activation/production, stability assessment, triangulation and reinitiation, activation/production reassessment, retriangulation process. Following completion of a task, each of the systems «judges» the impact of the sequence of events of which that task is composed by entropic guidelines inherent in that system. If stability is favored by additional activity and/or product of the network, a productive cycle will be initiated at the optimum time to favor additional systematic stability. If stability is optimum, a non-productive or minimally productive cycle will be initiated. This will appear as a relative refractory period during which systemic response will be damped; a period with low or no output of the unneeded products of the network. After that cycle has been traversed, entropic states and relative systematic stability will demand either initiation of another nonproductive cycle «marking time» or a more actively productive cycle, depending upon the needs for network stability and/or product.

This limit cycle model can be employed to aid accurate description and prediction of cyclical chemical reactions; enzymatic reactions; organ/system electrical conductance patterns; calcium flux-based electrical frequency and amplitude modulated intercellular communication; regular alternations of tissue and organ function and refractoriness; and complex temporally optimized cyclical organismic, ecosystem and societal behavior. The regularly, undulating responsive/refractory model of biodynamic behavior clearly favors stability and economy. The stuttering progression to a new state caused by the cyclical readjustment of networks moves the system gradually; to the right or left, up or down; allows time for accurate sensing of a new state, for retriangulation within the new state and re-response to it. The economy of having the production of one cycle available to trip a second set of product dependent cycles so that important metabolic tasks may be compartmentalized both temporally and spatially is essential to the efficiency of any biochemical process. Escape from this sort of temporal ordering has lethal consequences.

Casual observation of biologic time structure reveals highly complex and inapparently regular biovariability. Closer inspection, however, reveals a multifrequency resonance structure. Certain fundamental frequencies tightly tied to external or internal environmental regularities and their interacting harmonics combine to display a richly complex yet largely predictable time structure. The internal genetically determined circadian and menstrual cycles are a fair example of two primary rhythms tightly tied to internal genetic time-keeping mechanisms essential for organismic and species survival, respectively. Each cycle is also influenced by environmental cycles, the circadian very strongly and the menstrual cycle somewhat less strongly. Light/dark cycle changes can lead and shift endogenous circadian cycles and circadian cycle shifts can impact menstrual cycle regularity. It is not really known to what extent dietary, behavioral or pharmacologic interventions reproducibly change or regulate the inadequately studied yet powerful and important menstrual cycle. The consideration of the modulation of each rhythm by the other is a worthwhile exercise relevant to the general consideration of multifrequency rhythmic interactions. Depending upon variables of interest, other interacting frequency ranges must be concurrently considered. For example, using pulse time structure for evaluation of sympathetic/parasympathetic balance requires quantitative consideration of the fundamental heart beat frequency, the respiratory frequency, the fundamental frequency of the interaction of these two rhythms (the respiratory sinus arrhythmia), the overlying slightly lower frequency baroreceptor component and circadian heart rate variability. When analyzed quantitatively, each of these frequency ranges has information relevant to the stability and health of the cardiopulmonary systems. Quantitatively analyzing all four frequency rhythms and their interactions has considerably greater power.

Quantitative chronobiology determines boundaries between the predictable and the stochastic biovaria-

bility. The determination of when complexly interacting deterministic systems become chaotic has extreme utility to a variety of medical problems such as the study of electrical destabilization of cardiac conduction as well as predicting and understanding seizure activity in central nervous systems. The true understanding of frequency and amplitude modulated calcium flux-based electrical intercellular communication is also a challenge in the quantitative analysis of high frequency time structure that when mastered will allow decoding of the cellular vocabulary, grammar and syntax. The biodynamics of interacting multifrequency time structure is almost never stochastic, although it is usually complex. It can become stochastic. This is the node at which complexly organized behavior ceases its predictable regularity and becomes chaotic. Important premonitory information about the relative stability of biological systems will be rapidly and non-invasively obtained by quantitative analysis of the resonance structure long before the break point of fatal chaotic system behavior. Overall, a picture is emerging across disparate fields; from cardiac and neural electrophysiology and to endocrinology, carcinogenesis and pharmacology, that a dampening of resonance structure amplitudes precedes and predicts serious systematic dysfunction or «state change». That is, any system «under stress» flattens its resonance structure prior to a significant state changes. A loss of multifrequency biovariability of calcium flux, heart rate, respiratory pattern, EEG waves or endocrine function indicates that a potentially remediable breakdown of the system is imminent. These observations also raise the possibility that if that biovariability can be restored, an adverse state change that their flattening portends might be prevented. Accurately reading resonance structure stress is required for restoration of the system to its optimal degrees of freedom while tried therapeutic re-entry is required to stabilize the resonance structure and allow avoidance of undesired and unfavorable state changes.

A fundamentally novel conclusion flowing from an understanding of the biodynamics of organismic chronobiology is the appreciation of the essential non-linearity of all biosystems at every level of temporal organization. Stimulus-response relationships are different at different stages in the cycle(s) of interest. Response resultant from a stimulus can be both quantitatively and qualitatively different. While resonant time structure favors biological economy and systematic stability, it has an additional benefit to all living systems, increasing their flexibility and their vocabulary, grammar and syntax by some substantial order of magnitude. Only when the resonating non-linear reality of biological systems has been fully incorporated into strategies for the prevention, diagnosis and treatment of disease and the optimization of wellness, will the advances of the other medical revolutions be fully implementable. The practical implications of the ultradian/circadian/infradian temporal coordination of all biological processes are beginning to come into focus. Optimization of the temporal control of drug delivery is a challenge derived directly from the existence of this biological time structure that can no longer profitably be ignored.

## Some practical findings

In anticipation of the development of clinical chronotherapy and in order to pick clinical test times for doxorubicin and cisplatin trials, two large studies were performed on rats bearing a transplanted plasmacytoma [1]. The circadian timing of each of two anticancer drugs given at precisely equal dose intensities was expected to improve therapeutic benefit over conventionally given (time-unqualified) treatment. In each chronotherapeutic study, maximal benefit and minimal toxic effects were found when cisplatin was administered in the middle of latter part of the daily activity (dark) span, while doxorubicin was administered near the end of the daily resting (light) span for these nocturnally active rodents living on a 12h/12h or 8h/16h light/dark schedule. This was true whether doxorubicin or cisplatin was given first and whether there was a lag of only a few hours or a few days between the admistration of these two agents.

## Advanced ovarian cancer

Animal studies have indicated that the time of administration of adriamycin and cisplatin, two widely used anticancer drugs, has a profound effect on their toxicity. This effect in cancer chemotherapy was studied in 31 patients with advanced ovarian cancer. Patients received at least eight monthly courses of adriamycin that were followed 12 h later by cisplatin, with adriamycin randomly administered at either 6 a.m. or 6 p.m. The results show that in the group receiving adriamycin in the evening and cisplatin in the morning: twice as many patients required reductions in dosage and delays in treatment; four times as many treatments had to be delayed; drug dosages had to be modified downward three times as often; and, even with more dose attenuation and treatment delays, treatment complications were still about two times more common as in the group receiving adriamycin in the morning and cisplatin in the evening. These findings show that the circadian stage at which anticancer drugs are given to patients should be carefully considered [2].

## Kidney cancer chrono-chemotherapy

For decades oncologists have been frustrated by the relentless biology of metastatic kidney cancer that has resulted in an expected 3-6 month survival for its 12,000 to 15,000 annual victims. Although

the disease's behavior, in aggregate, has been highly predictable and uniformly dismal, rare exceptions of prolonged survival or spontaneous regression indicate to the open minded, that the overall balance between cancer and host can make a critical difference in determination of the ultimate outcome of this fascinating disease. This hopelessness associated with the diagnosis of metastatic renal cell carcinoma has been cracking during the last 5 years. Immunomodulation with interferons or interleukin-2 have clearly been shown to favorably modulate the host tumor balance in a real minority of treated patients. Our approach of carefully examining a novel way of administering an antimetabolite shown previously not to have been useful in this disease, has also yielded interesting results. FUDR is a pyrimidine nucleotide analogue which damages cells (following conversion to FdUMP) only during the process of DNA synthesis by binding to thymidylate synthase. RCC is usually a very low growth fraction tumor, therefore, we have employed a long term continuous infusion for 14 out of every 21 to 28 days. Normal gut and bone marrow cells synthesize DNA most actively in the later half of the night and first half of the morning, therefore, we have modulated the FUDR infusion so that most of each day's continuous daily dose is given in the early evening and first half of the night. With this approach, we have been able to increase the average safe dose intensity by 50%, diminish toxicity and control progressive RCC in the majority of patients treated. Objective responses have occurred in 29% of patients, 40% of whom had failed prior therapy. This therapy, is accomplished entirely, in an outpatient setting with a single visit every two weeks and minimal toxicity, or lifestyle disruption. Recent results indicate that interleukin and interferon circadian timing are far more critical than is the timing of FUDR. In fact, in our hands using a mouse tumor model system, improper IL-2 timing is as likely to result in tumor growth stimulation as proper timing is to result in tumor growth control [3, 4].

In addition to these findings with IL-2, it has been recently discovered in murine systems that the circadian timing of recombinant growth factors such as erythropoietin and granulocyte colony stimulating factor is critical to the bioactivity of these molecules [5].

### Infusional chrono-chemotherapy

The recent history and, to some extent, the rationale for long-term infusional chemotherapy and for optimally timed circadian chemotherapy infusions have intersected and been intertwined. The resistance to adoption of these simple logical techniques for favorably modifying the toxic-therapeutic ratio of cytotoxic therapy has also been shared. It is becoming clear that the protracted infusion of many agents diminishes toxicity, allows higher dose intensity, and increases the likelihood of tumor response as well as the duration of that response in both relatively refractory and sensitive tumor types [6].

Many, if not most, of the cytotoxic agents comprising our armamentarium affect cells that are actively dividing more profoundly than cells that are resting between cell division cycles. Some of the most effective of these cytotoxic agents not only have increased cytotoxicity to cells that are actively dividing, but some of these agents act only upon cells in certain specific cell cycle phases of this active division process.

For most human cancers and for all of the common solid tumors of human beings for which tumor labeling indices have been determined, the proportion of cells within cancers that is actively engaged in cell division cycle has been shown to be relatively small. Even in rapidly expanding malignant cell populations of hematologic cancers the proportion of actively dividing cells is small at any given time.

For most common cancers this proportion is only a small fraction of these values. Protracted infusional chemotherapy then provides biologically relevant concentrations of drug over long spans so that the agent is available to a greater proportion of dividing cells in aggregate. Drugs that act only during certain specific phases of the division cycle magnify this problem in population selectively. For example, for drugs only acting during the DNA synthetic phase of the cell cycle, the likelihood of significant cell kill from a brief exposure is obviously small. Logic compels us to then administer most cell cycle phase-specific agents generally more active against dividing cells either very frequently or continuously for spans that will permit exposure of a greater proportion of these sensitive cells.

Infusional chronotherapy extends these simple principles only slightly. It does so merely by acknowledging the essential nonlinearity of resonating living systems. Quite simply put, all potentially drug-sensitive cells in normal tissues within the day, at least in part, because they are not actively dividing at all times within the day. The cells in these tissues initiate the cell division process nonrandomly during the circadian cycle. The population dynamics of cell division and associated cell sensitivity to cell cycle - and cycle phase - specific cytotoxic agents in all relevant target tissues for drug toxicity are tightly organized within the circadian cycle of the human being. The compelling logic for the use of long exposure to improve tumor cell kill can therefore be fine tuned by weighing this prolonged exposure so that most of each day's continuous infusion dose is given during that part of the day when toxicity to normal tissue is least profound. For S-phase active agents this would correspond to the time of day when the least DNA synthesis is going on in the tissues which most

clearly limit the effective dose intensity for these agents. For most S-phase-active drugs, these tissues typically include bone marrow and gut epithelium. Complementarily, most drugs should best be given during the time of day when most of the daily DNA synthetic activity was occurring in tumor tissues. Because of the necessity for multiple around-the-clock biopsies it is extremely difficult to accomplish studies of the cell cycle distribution and DNA synthetic capacity in the relevant tissues and cancers of normal human beings. This is, however, a growing body of data that has attempted to do just this.

Normal human (medical student) bone marrow DNA synthetic capacity and mitotic index was demonstrated by Alvin Mauer, in 1965 [7] to be predictably different at different times within the day. Mauer's direct bone marrow sampling confirmed the observation of Killman *et al.* [8] of the circadian rhythm of human colony-forming units in culture in bone marrow, which Ross later confirmed in circulating blood. Recently, a yeoman study by Smaaland *et al.* [9] who sampled bone marrow around the clock for more than 20 normal individuals and cytofluorometrically examined the cell cycle phase distributions, again confirmed the fact that the DNA synthetic capacity of normal human bone marrow, is greatest between midnight and mid-morning while very little DNA synthesis occurs later in the day and early in the night. These data clearly indicate that if bone marrow is the dose-limiting target for an S-phase-specific cytotoxic agent that, in the absence of important alternative targets to drug toxicity, more damage will be done when most of the daily infusion is given in the early morning hours. Clinical studies of drugs that damage bone marrow have demonstrated clear circadian dependency to drug-induced cytopenias [2]. Clinical tests of S-phase-active cytotoxic agents with marrow as a major target of drug toxicity are required and are currently ongoing [10].

The human gut epithelium is a toxicity target of rapidly growing importance. As we prolong the duration of infusions, as we have more and more hemopoietic growth factors available, and as we develop and refine bone marrow transplantation strategies, the relative importance of epithelial gut toxicity has crescendoed to the point where it is becoming the leading contributor to treatment-induced death in a variety of therapeutic settings.

DNA synthesis in the gut epithelium of mouse and rat has been known for decades to be highly organized within the day [11]. Recently, however, this finding was courageously extended by Buchi *et al.* [12] to a thorough investigation of the human large bowel epithelium. In 24 individuals (10 fasting for 48 h and 14 eating regularly) two or three biopsies of the colorectal mucosa were obtained every 3 h for a maximum of 27 consecutive h. DNA synthetic capacity of these cells was then evaluated by measuring their ability to incorporate tritiated thymidine. It was found, as in the bone marrow, that human gut epithelium DNA synthetic capacity was not randomly distributed throughout the day. There is far greater average activity in the samples obtained in the morning hours than at opposite times of the day. This pattern was substantially and identically rhythmic whether subjects had fasted or eaten regularly, although the 24 h mean DNA synthesis level was lowered by fasting. These results indicate that infusions of S-phase-active drugs such as 5-flourouracil (5FU) or 5-fluorodeoxyuracil (FUDR) with or without concomitant folinic acid would be predicted to be much more gut toxic if most of the daily dose was delivered in the morning hours. Conversely, these agents should be far safer when most of the daily infusion is administered in the evening. This is precisely what has been found by a number of investigators. The most thorough work has demonstrated, for example, that the maximum safe dose intensity of FUDR infusion can be increased by an average of about 50% and in some individuals by more than 200% by administering most of the continuous infusion in the evening hours. The only significant toxicity of FUDR infusion, which is epithelial gut damage, is, on average, far less severe even at these much higher doses than when the drug is given by a standard continuous constant rate infusion [13]. Similar data for 5-FU and 5-FU plus folinic acid have also more recently become available [10].

Very few data are available to allow us to determine whether tumor cytokinetics are coordinated within circadian time. The few examples that do exist, however, seem to indicate that they may, to some extent, be governed by circadian growth control mechanisms [14, 15]. Other data clearly indicate that tumor susceptibility may be predictably different at different circadian stages regardless of whether or not cytokinetic circadian coordination exists. These sorts of non-cell cycle phase-related time-dependent differences often relate to circadian differences in the biochemical pharmacology of drugs in target tissue, which are also highly organized in circadian time [16-18].

These few examples indicate clearly that S-phase active cytotoxic chemotherapy must be present long enough to effect its cytotoxicity upon a meaningful number of malignant cells; that normal tissues are more or less DNA system is occurring within the sensitive population; that respecting this circadian time structure can result in safe elevation of dose intensity; and that, at least preliminarily, this simple and logical strategy can result in safer and more effective infusional cytotoxic chemotherapy. While it is clear that a higher dose intensity can be safely given by modulating the infusion according to time of day, it remains to be proven whether or not this will be translated into higher cure frequencies or better cancer control. Preliminary, clinical results do indicate, however, that this may be the case [11].

There is, in essence every reason to believe that, just as the strategy of long term continuous infusion favorably modulates the toxic therapeutic ratio of many agents, the optimal circadian shaping of these agents further improves benefit and minimizes toxicity. This article gives a few clinical examples of evidence to support this hypothesis.

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