Circadian Pharmacodynamics of Anticancer Therapies

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Circadian variation in drug metabolism and tissue sensitivity to drugs affects their activity and toxicity. A growing body of data suggests that therapy may be improved and toxicity reduced by administering antineoplastic agents at carefully selected times of the day. Here I briefly review molecular, cellular, and organismic time-keeping mechanisms as well as cytokinetic, pharmacokinetic, and pharmacodynamic data, which support the predictable and exploitable nonlinear dynamic relation between dose and effect that occurs each day.

**Indexing Terms:** chronobiology · fluoropyrimidines · doxorubicin · cisplatin · cytokines · pharmacokinetics

**Background**

In 1972, a paper in *Science* reported that the arrangement within a given day of 3-h doses of cytosine arabinoside (Ara-C) had a profound effect on the cure of mice inoculated with L1210 leukemia cells (1). This study was built on extensive prior work and demonstrated that all Ara-C toxicities are markedly dependent on the time in the day/night cycle at which the drug is administered (2). Together, the experiments showed unequivocally that, in mice, the timing of Ara-C administration predictably modulates its therapeutic index.

Surprisingly, nearly 20 years later, this simple hypothesis has still not been extended to clinical trials for human leukemia, even though the mainstay treatment for the most common deadly acute leukemias has remained Ara-C, used at higher and higher dose intensity with greater and greater toxicity (3). In the meantime, it has been shown that all anthracyclines, which are generally coupled with Ara-C to treat the nonlymphocytic leukemias, also exhibit a pronounced circadian time dependency in their pharmacology, toxicology, and efficacy in mice and humans (4–6). Furthermore, many combination chemotherapy studies, done in follow-up to the initial Ara-C study, have demonstrated that the addition of a second or third drug to the regimen seldom interferes with the enhancement in therapeutic index that results from circadian optimization of each drug (7).

My background of clinical experience with high-dose cytoxan in G.W. Santos’ pioneering bone-marrow transplant unit and with ultra-high-dose-intensity chemotherapy for small-cell lung cancer as a member of the National Cancer Institute solid tumor service made it impossible for me to ignore the likely clinical impact of diminishing the toxicity and increasing the efficacy of the drugs available by any means, including optimal circadian timing. A series of studies in rodents that led to the circadian optimization of the doxorubicin/cisplatin combination was critical to the development of clinical protocol (8). Randomized clinical studies sponsored by the National Cancer Institute subsequently revealed that toxicity of doxorubicin and cisplatin in humans depends largely on the time in the circadian cycle at which they are administered (4, 9). In patients with widespread ovarian cancer, optimal circadian timing resulted in safer administration of higher doses of drug and, in turn, a fourfold improvement in the 5-year survival rate of women with advanced ovarian cancer (10).

More recently, a shift in patterns of patient accrual and the concurrent availability of programmable, implantable drug-delivery devices have led to clinical studies of the time dependency of fluoropyrimidine pharmacology, toxicology, and efficacy. As with Ara-C, doxorubicin/cisplatin, and other anticancer agents, the fluoropyrimidine story began with rodent experimentation. Studies with mice in the early 1980s revealed that the LD₅₀ of 5-fluorouracil (5-FU), a mainstay of solid tumor treatment, is markedly and reproducibly higher when the drug is given in the sleep span (11, 12). Peters et al. confirmed this early work and extended it to show that nonspecific 5-FU toxicity and 5-FU efficacy against a murine colon carcinoma are each dependent on circadian timing (13).

**Fluoropyrimidine Chronotherapy**

The programmable automated delivery systems initially available for clinical cancer treatment were of small volume, and thus required highly concentrated drug. In anticipation of new clinical chronobiological chemotherapy studies, fluorodeoxyuridine (FUDR), a more highly concentratable fluoropyrimidine, was studied in mice and rats. Chronotoxicology studies of intravenous and intraperitoneal bolus FUDR administration in mice revealed that the safest time for this drug was several hours earlier in the day than that for 5-FU (14). The highest LD₅₀ (lowest toxicity) occurred reproducibly late in the daily activity span. FUDR has an extremely short half-life, and hence must be administered by constant infusion. Extensive continuous infusion studies on Fisher 344 rats carrying transplanted fluoropyrimidine-sensitive 13762NT breast adenocarcinomas revealed that continuous infusions weighted for delivery in the late activity phase and first half of the usual sleep span are reproducibly far less toxic and significantly more effective than constant-rate infusions or infusions...
that deliver peak concentrations of the drug at other
times of the day (15).

With this preclinical information, a series of random-
ized clinical studies was initiated to investigate whether
the systemic and intrahepatic toxicities of FUDR could
be lessened and the dose intensity safely increased by
administering the drug late in the cancer patient’s usual
daily activity span (16). Thus far, we have shown that
optimal drug timing offers a clinical advantage for sys-
temic intravenous FUDR infusion in patients with met-
astatic renal cell cancer (17) and for intraarterial FUDR
infusion in patients with liver metastases from colorectal
cancer (18). Levi et al. have also demonstrated a clinical
advantage to shaping 5-FU infusion in patients with
widespread colorectal cancer (19).

We and other investigators have begun to define the
mechanisms underlying the circadian time dependency
of fluoropyrimidine toxicity and anticancer activity. Of
potential importance is the way drugs are handled by
the organs primarily responsible for catabolism or ex-
cretion (drug pharmacology), the way drugs are handled
by the targets of toxicity (biochemical pharmacology),
and, correlatively, the cell-cycle phase of the target cells
at the time of drug exposure (circadian cytoke
ics).

Figure 1 demonstrates some of the advantages of opti-
mal circadian shaping of FUDR continuous infusion in
tumor-bearing rats and in patients with metastatic re-
nal cell carcinoma.

5-FU Chronopharmacokinetics

The circadian-dependent pharmacology of continuous,
constant-rate 5-FU infusion, as measured by rhythmic
fluctuations in plasma concentrations of the drug
throughout the day, was first demonstrated by Petit et
al. in patients also receiving cisplatin (20) and later by
Harris et al. in patients receiving only 5-FU (21). Each
group noted marked variations in pharmacokinetics at
different times of the day, although the timing of peak
FU concentrations differed between these two groups
of patients. The difference may be related to the fact that
the patients in the first study received cisplatin at a
fixed time of day before each 5-day infusion of 5-FU,
whereas the patients in the second study received only
5-FU and for much longer spans. Nonetheless, both pa-
ners clearly demonstrate that there are large, within-
study and cross-subject reproducible differences in
plasma concentrations of 5-FU at different times of day
during a continuous, constant-rate 5-FU infusion.

Fig. 1. Circadian schedule dependency of FUDR-induced tumor control in a rat breast cancer model system (left panel) contrasted with
the circadian schedule dependency of FUDR measures of toxicity in human patients (right panel).

Each member of these groups of rats and cancer patients was randomized to receive the identical dose of infusional FUDR chemotherapy. In one group, rats or
patients received continuous constant-rate infusion (88) or infusions peaking late in each daily activity span and early in the usual daily sleep span (89). In
the circadian-based schedules, 88% of each day’s dose was given during these 8 h, 15% in each abutting daily quadrant, and 7% of each daily dose near daily ar
ing. In the rat, FUDR toxicity was affected by the circadian shape of the infusion (data not shown) and tumor shrinkage was enhanced by optimal circadian FUDR
infusion shape. In 30 cancer patients, randomly assigned to one or the other infusion pattern, toxicity was markedly diminished by optimal timing (data not shown).
This circadian toxicity dependence resulted in 8 times more frequent treatment delays of ≥1 week and 8 times more frequent dose reductions of ≥20% than when
constant-rate infusion was used. This also resulted in being able to safely increase the average dose intensity by circadian infusion by at least 50% (from 0.4 to
0.65 mg/kg per week for FUDR). Data from von Roemeling and Hrushesky, J Clin Oncol 1995;13:1710–9.
Circadian Fluoropyrimidine Biochemical Pharmacology

Work by Tuchman et al. (22) and later by Disio et al. (23) demonstrated the importance of dihydropyrimidine dehydrogenase (DPD) as the rate-limiting enzyme involved in the breakdown of 5-FU to nontoxic metabolites. When human mononuclear cells from peripheral blood were isolated around the clock in seven subjects, Tuchman et al. found that DPD activities were much higher just after usual sleep onset than at other times of the day (24). In their elegant ex vivo rat liver perfusion study, Harris et al. (25) demonstrated unequivocally that 5-FU clearance and metabolism by the liver are critically circadian-stage dependent: Eight times as much 5-FU is extracted per unit time from perfused rat livers removed in mid- to late sleep compared with those removed in mid-activity phase (25). Data from Tuchman et al. showing that the concentrations of human mononuclear DPD peak during sleep (24) are consistent with the results of the perfusion experiments in rodents. In aggregate, these data suggest that a prominent circadian rhythm in DPD activity, exhibited both by organs of metabolism and by cells more peripheral to 5-FU catabolism, may be responsible, at least in part, for the circadian dependence of 5-FU toxicity in rodents and humans. This rhythm perhaps also accounts for the circadian dependence of the drug’s anticancer efficacy documented in rodent models and, more recently, suggested by work with cancer patients.

The circadian pharmacology and biochemical pharmacology of FUDR are less clearly established than those of 5-FU, in part because the former drug is administered in much lower (milligram) amounts and requires more sensitive analytical methods. Moreover, these two fluoropyrimidines are to some extent interconvertible. In preliminary experiments, von Roemeling et al. observed a twofold circadian difference in the convertibility of FUDR to 5-FU, indicating that FUDR pharmacodynamics may depend on the circadian DPD rhythms at some times of the day but not at others (26). The activities of other enzymes of importance in the activation of FUDR to fluorodeoxyuridyl monophosphate (FDUMP)—i.e., DPD, uridine phosphorylase, and thymidine phosphorylase—have each been shown to be subject to circadian rhythms in mouse liver (27); however, assessment of the exact contribution of these enzymes to the crisis, high-amplitude circadian rhythm in FUDR toxicity and efficacy requires further work in murine and human systems. Together, these preclinical and clinical results with fluoropyrimidines indicate that circadian time structure in the organs of catabolism and excretion and the biochemical enzyme activity rhythms in normal or malignant target cells may each be critically important in determining the optimal time of day for bolus drug administration or the optimal circadian shape of continuous-infusion fluoropyrimidine therapy. Because the metabolic processes necessary to catabolize these two closely related drugs are different yet related, and because the intracellular targets are overlapping but to some extent distinct, the two agents when given by intravenous bolus have distinct optimal circadian timings that are nearly a third of a day apart (14). However, all therapeutic strategies that focus the fluoropyrimidine on thymidylate synthase (TS) result in an optimal time of day in the first half of the daily sleep span. These strategies include the use of FUDR, which is metabolically closer to FdUMP than is 5-FU; the long-term infusion of 5-FU, which may also to some extent favor the FDUMP pathway and result in more efficient TS inhibition; and the addition of leucovorin to 5-FU, which also increases fluoropyrimidine-induced inhibition of TS by stabilizing the FDUMP/TS complex. These three therapies result in an optimal circadian timing during the first half of the daily sleep span and focus on TS blockade, indicating that TS blockade may be least effective in normal gut tissue at this time of day. This is consistent with observation of less gut damage from fluoropyrimidine given in the first half of the daily sleep span.

Normal Tissue Circadian Cytokinetic Coordination

In addition to the circadian patterns of crucial enzyme activities, circadian patterns of cytokinetic activity in malignant and nonmalignant tissues damaged by fluoropyrimidines may be of equal importance in explaining the circadian pharmacodynamics of these drugs. Both 5-FU and FUDR are most active against cells undergoing DNA synthesis. DNA synthesis in all tissues studied throughout the circadian cycle is nonrandomly distributed throughout the day (28). Depending on dose and duration of infusion, the gut, skin, and bone marrow are the primary targets of fluoropyrimidine toxicity. Human skin (29), bone marrow (30-33), and colorectal mucosa have each been found to exhibit marked circadian rhythms in DNA synthesis (34). When FUDR is infused such that lower amounts are delivered during early morning hours, the clinical toxicity is markedly diminished and dose intensity can be safely increased. In humans, the dose-limiting target of FUDR infusional toxicity is the colorectal mucosa. Serial biopsies of rectal mucosa every 3 h for 24 h from 24 human volunteers in both the fed and fasted states reveal that in vitro uptake of [3H]thymidine (presumably reflecting DNA synthesis) is much greater in colonic epithelial cells removed between midnight and 0400 than that taken later in the day and evening (35). This circadian-stage coincidence of minimal [3H]thymidine uptake and minimal FUDR toxicity to colon epithelial cells is intriguing and warrants further study.

Continuous Fluoropyrimidine Constant-Rate Infusion

The top panel of Figure 2 depicts the circadian time structures of the activities of certain key enzymes relevant to the cytotoxic effects of fluoropyrimidines. Figure 2 also relates the implications of these rhythms to fluoropyrimidine metabolism during a continuous infusion of 5-FU and (or) FUDR at a constant (zero-order) rate. The activity of thymidine phosphorylase, an important enzyme in the conversion of FUDR to 5-FU, remains almost constant throughout the day. This results in a nearly constant rate of conversion of FUDR to 5-FU.

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Rhythmic 5-FU catabolism. In contrast, the activity of DH), which catabolizes 5-FU, is highly rhythmic throughout each day. The wanning of DPD activity during the daily activity span results in an accumulation of 5-FU during the day. The endogenous circadian increase in DPD activity during the evening results in daily decreases of 5-FU concentrations during the evening. In other words, the bioavailability of 5-FU waxes and wanes during the day. During FUDR infusion, the near constant thymidine phosphorylase activity does not modify the effect of rhythmic DPD activity on the rhythmicity of 5-FU concentrations. The effective concentration of fluoropyrimidine species is therefore higher during the morning hours, when less FU has been removed.

Rhythmic FUDR anabolism. The activity of thymidine kinase, which converts FUDR to FDUMP, which in turn binds to and inactivates TS and thereby blocks thymidylate and subsequent DNA synthesis, is not constant throughout the day. This enzyme has far greater activity in the morning hours when the concentrations of fluoropyrimidine species are greatest. This thymidine kinase rhythmic activity theoretically results in an even greater amplitude in daily FDUMP availability. The middle panel of Figure 2 demonstrates the effects that these circadian patterns of enzyme activity have on the concentrations of fluoropyrimidines; it also depicts the theoretical effect this time structure may have on intracellular FDUMP concentration during a constant-rate continuous infusion of fluoropyrimidine.

Rhythmic gut cytokinetics. The amount of target enzyme (TS) activity covaries with the DNA synthetic capacity of the tissue. TS is, in fact, in critical supply only during the process of DNA synthesis. The bottom panel of Figure 2 demonstrates the experimentally determined circadian pattern of DNA synthetic activity in the two human tissues that are most dominantly damaged by fluoropyrimidines: gut mucosa and bone marrow; indeed, DNA synthetic activity in these tissues cycles prominently within each day. Much more DNA synthesis occurs in the morning, during the first half of the daily activity span. The daily timing of high DNA synthetic activity in both of these critical tissues occurs when DPD activity is lowest and thymidine kinase activity is highest, coinciding with the time of day of the highest DNA synthesis and the time associated with the greatest need for TS activity. These temporal coincidences mean that a zero-order infusion of FU or FUDR can be predicted to result in a high-amplitude circadian rhythm in the active intracellular TS blocker, FDUMP. However, this remains to be proven experimentally. This theoretical peak in daily FDUMP availability would happen at the time associated with the highest daily synthesis of DNA in gut and bone marrow and therefore the time of the greatest need for TS activity in gut and bone marrow and the time of highest tissue susceptibility. The time-of-day-dependent toxicology predicted by the arrangement in time of these critical enzyme activities has already been clearly demonstrated in mice, rats, and humans, as discussed earlier.
**Useful predictions.** Changing the 5-FU infusion shape to give more drug in the evening hours and less in the morning hours would be thereby expected, for the same reasons, to result in predictably less interference with gut and bone marrow synthesis of DNA and less toxicity to normal tissue. This too has already been demonstrated in several species, including humans.

**Tumor Circadian Cytokinetic Dynamics**

Few data are available to evaluate whether spontaneous human malignancies are cytokinetically coordinated on the circadian time scale. The most thorough evaluation of this difficult question has been accomplished in patients with ovarian cancer. Klevecz et al. (36) sampled cells washed from the peritoneal cavities of a large number of women, every 2-4 h for as long as 4 days. Using sophisticated cytofluorometric techniques, these investigators found circadian coordination of the proportion of both malignant epithelial and nonmalignant mesothelial cells synthesizing DNA. A greater proportion of malignant cells was actively synthesizing DNA in the morning hours, whereas the proportion of nonmalignant cells engaged in DNA synthesis was greater in the evening hours (36). In another study, a smaller number of patients with non-Hodgkin lymphoma underwent thin-needle aspiration of tumor masses every 4 h for at least 24 h. A circadian rhythm in the proportion of cytofluorometrically determined S-phase cells was found in this population, with most individuals exhibiting large time-dependent differences and with peak DNA synthesis activities clustering around midnight and the very early morning hours (37, 38). Serial biopsies of tumor and normal skin in one of my patients with widespread cutaneous epidermoid carcinoma revealed high-amplitude circadian rhythms in the mitotic index of her tumor cell population with precisely the same circadian phase as her normal skin. Mitotic indices of tumor and normal tissues were identical at the times for the daily lowest values, whereas tumor cell mitoses were manyfold more frequent at times in the day associated with the usual highest daily mitotic activity (W.J.M. Hrushey, E. Haus, D. Lanning; unpublished data). These limited series of data suggest that the cytokinetic activity of tumor tissues is likely to be coordinated in circadian time and that there may well be windows in circadian time when cell-cycle-stage-specific attacks on these cells may be more effective or less effective.

Predicting the optimal time of day to treat cancer cells with cell-cycle-phase-specific agents may depend more on knowing the cytokinetic time structure of the cells/tissues of malignant cell origin than on whether those cells are malignant. Asking whether malignant cells participate in circadian cytokinetic rhythms may be less profitable and less relevant than asking to what extent malignant cells participate and determining how their cytokinetic rhythms differ in phasing and (or) amplitude from the analogous rhythms in the normal tissues that are prominently damaged by the drugs we wish to use to treat that particular tumor type.

Taken together, the preclinical and clinical data defining optimum timings of 5-FU and FUDR administration are compelling. The time-dependent differences in toxic-therapeutic ratios of these drugs are substantial and we are now beginning to understand the biochemical and cellular mechanisms that contribute to these differences.

**Implications for Clinical Trials with Cytotoxic Drugs**

A complete understanding of circadian control mechanisms should not be a prerequisite for incorporation of these ideas into clinical trials. Clearly many advances in the chemotherapeutic control of human malignancy have been gained without a sophisticated understanding of the mechanisms of action of each drug in effective chemotherapy regimens. In my view, our present knowledge of circadian pharmacodynamics is sufficient to enable us not only to optimize the effectiveness of the chemotherapeutic agents currently in use but perhaps also to expand the repertoire of effective anticancer treatments. It is daunting to realize that scores of anticancer drugs discovered in murine screens and subsequently rejected in phase I trials because of unacceptably high toxicity to humans were recommended for clinical study on the basis of murine toxicology and efficacy studies performed during the animals' usual sleep span and were later rejected clinically on the basis of studies routinely performed during the activity span of the cancer patients tested.

**The Future**

As newer therapeutic approaches are developed with growth factors and other genetically engineered proteins, temporal questions become ever more important. We have already found, for example, that (a) the LD50 for tumor necrosis factor varies by the magnitude of one logarithm and its curative efficacy varies sevenfold, depending on the time of day it is administered (39); (b) recombinant human erythropoietin increases reticulocyte responses 10-fold if it is given at an optimal vs a suboptimal time of day (40); and (c) recombinant human interleukin-2 either doubles the number of splenocyte (putative) natural killer cells or fails to increase it, depending on whether the drug is given during the rest or activity phase of the murine circadian cycle (41). More important, perhaps, circadian timing has been repeatedly shown to be responsible for reproducible tumor growth enhancement if interleukin-2 is given at the wrong time of day. The identical dose and schedule at an opposite time of day is associated with reproducible tumor shrinkage (unpublished data). A wide variety and rapidly growing number of programmable drug-delivery strategies are being perfected and applied to the practical challenges of circadian-optimized treatment (42). We can no longer afford to deny either the existence or the importance of the circadian time structure of living organisms if we are to optimally apply both established and new technologies to effective cancer control.
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