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CHRONOBIOLOGY

Implications for cancer chemotherapy

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Experimental studies have documented that both the toxicity and the antitumor activity of many cancer drugs are time dependent. Early clinical trials have confirmed this observation for several drugs. The basic concepts of chronobiology and its application to pharmacology are reviewed. As an example, clinical trials of circadian fluoropyrimidine delivery are reviewed. Other clinical results are presented in table form. The mechanisms pertinent to the circadian time dependence of fluoropyrimidines are discussed.

The toxicities of at least 20 chemotherapeutic agents have been shown in murine systems to be dependent on the time of day (1). The anticancer efficacy of many of these agents, either given alone or in combination, has also been shown in experimental studies to have circadian time dependence (2, 3). This reproducible temporal variability in antitumor effect and in normal tissue toxicity can be explained in part by three important observations. First, experimental and clinical studies have documented that the pharmacokinetics of many anticancer drugs (and most other drugs studied), show consistent and reproducible circadian variation depending on the time of their administration (4–8). Second, most normal tissues are reproducibly either more or less sensitive to the effects of drugs at specific times of day (9–11). Finally some tumors exhibit similar rhythmic susceptibility patterns, while others seem to vary little during the circadian cycle (12–15). These circadian rhythmic variations in drug pharmacokinetics and tissue susceptibility have been exploited to select a time for treatment that results in an increased tumor-cell kill and reduced toxicity in several murine systems (2). Clinical data are now available which show that the dosing time of 5-fluorouracil (16–18), floxuridine (19–21), 4′-O-tetrahydropyranyl-doxorubicin (22) and oxaliplatin (17, 23) markedly affects toxicity and safely achievable dose intensity (24). The toxicity of combination chemotherapy with cisplatin and doxorubicin has been shown to depend upon the time of administration (25–27). Whether this scheduling strategy can improve tumor response and overall survival is the subject of ongoing prospective randomized clinical trials. One such trial has indeed shown improved response rate with time modified delivery of chemotherapy (28).

More recently, experimental studies have documented even more pronounced circadian stage dependence for the activity and toxicity of several biologic response modifiers including erythropoietin (29), granulocyte-colony stimulating factor (30), interleukin-2 (31–33), tumor necrosis factor (34), and interferon (35–37). This remains to be confirmed in clinical trials.

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Chronobiology

Chronobiology is the quantitative study of the temporal relationships of biologic phenomena. Even the most superficial quantitative study of biodynamics demonstrates that biophysical and biochemical processes vary with respect to time in a regular and predictable periodic manner across several rhythmic frequencies (38, 39). Endogenous biological rhythms have been demonstrated at all biological levels,

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from yeasts and nucleated unicells to man, and at all levels of biological organization (38–40).

The existence of a molecular time-keeping mechanism, 'clock gene', was first inferred for Drosophila melanogaster by Konopka & Benzer (41), which has been named per (period). In several other species (neurospora, chlamydomonas) single gene mutations have now been shown to alter such basic clock properties as period length, light entrainability and temperature compensation (42, 43). A mammalian clock gene mutation has subsequently been found, that dramatically alters the period of the circadian locomotor rhythm of golden hamsters (44).

There is evidence to suggest that the suprachiasmatic nucleus (SCN) of the hypothalamus is a site of critically important circadian pacemaker cells in mammals (45). The most definitive experimental evidence for primacy of the SCN in circadian organismic time keeping demonstrates that circadian rhythmicity can be restored to SCN-lesioned arrhythmic hamsters by implantation of fetal brain tissue containing SCN cells (46–49). More recently Ralph et al. (50) have demonstrated that small neural grafts, from the SCN of normal hamsters and τ-mutant hamsters, restored circadian rhythms to arrhythmic animals whose own nucleus had been ablated. The restored rhythms always exhibited the period of the donor genotype. The proto-oncongene c-fos may be a molecular component of the photic pathway necessary for entrainment of mammals to the light/dark cycle (51, 52). Light pulses during the subjective night in hamsters caused a rapid increase in the SCN levels of c-fos, c-fos-mRNA and the immediate early protein product. This occurred only during the subjective night, at circadian times when photic phase shifting of activity could be induced (53).

At least three major biologic rhythms have been defined, which correspond to obvious periodic changes in the environment: The circadian rhythm (20–28 h, the solar day); the circatrigintan rhythm (30 ± 7 days, the lunar month); and the circannual rhythm (12 ± 2 months, the year). Of each of the biofrequency domains, the circadian rhythm has been most thoroughly investigated. Moore-Ede et al. have reviewed its' potential importance in health and disease (54, 55). The basic properties of biological rhythms are similar in plants and animals (38). The rhythms are endogenous and genetic in origin, persist without time clues, and are regularly influenced by cyclic variations of certain environmental factors called synchronizers. When precisely measured under constant conditions, the endo-genous circadian period lengths of the various species are not precisely 24 h. When removed from time cues human beings usually have a free running period length of somewhat more than 24 h but less than 25 h (56). Thus if their circadian pacemakers were not reset by their daily schedule, the timing of their endogenous rhythms would be delayed with respect to clock time each day. In man and many other species, the most powerful synchronizers of the circadian rhythm are the diurnal alternation of light (activity) and darkness (rest) and our 24-h life routine.

There are two general categories of circadian organization which bear most directly upon the practice of oncology. These are the circadian aspects of drug handling and the circadian organization of cell division in normal and malignant tissues.

Circadian variation in drug handling and effect

A better understanding of the temporal changes that have been described in drug effect as a function of timing can be achieved by considering three important concepts. First, experimental and clinical studies have documented that the pharmacokinetics of many anticancer drugs show consistent and reproducible circadian temporal variation depending on the time of their administration (4–8). The temporal variations that have been documented in drug absorption and distribution (57), metabolism (58) and excretion (59, 60) could explain this to a large extent. Hemodynamic circadian rhythms are also potentially relevant to drug delivery and metabolism. Studies in humans have documented a significant circadian variation in hepatic blood flow, with the maximum flow occurring at approximately 8 a.m. (61). This would affect the metabolism of drugs that exhibit hepatic blood flow-dependent clearance. Tumor blood flow, and therefore, drug delivery are twofold higher during the daily activity span of nocturnally active rats, and not influenced by modulators of normal hemodynamics, such as angiotensin II (62).

Second, most normal tissues are reproducibly either more or less sensitive to the effects of drugs at specific times of day (9). Sometimes the variable sensitivity can be explained and quantified in terms of bioperiodic changes in the concentration of receptors of a given system for a given drug (63–65). In other cases, a circadian variation in cellular defense mechanisms such as oxygen free radical defense mechanisms may play a part (66–69). Cell proliferation rhythms in the gastrointestinal tract and bone marrow are especially relevant to the oncologist, since these two tissues are the most common target tissues for toxicity from antineoplastic drugs. There is a marked circadian variation in cell proliferation throughout the gut mucosa, from the tongue to the rectum in mice and rats (70, 71). Similar rhythms have recently been documented in the gastrointestinal tract in humans (11), with the highest DNA synthetic activity each day between 5 to 9 a.m. each morning. The bone marrow is the most common tissue to limit dose intensity of the common anticancer drugs. The production of all types of blood cells undergoes strong regular temporal variations, and circadian and seasonal rhythms in blood cell production have been described (72). Recent studies of Smaaland et al. (10) upon a

group of 16 normal control subjects unequivocally confirm the earlier findings of Killman (73) and Mauer (74). The percentage of cells in DNA synthesis in the bone marrow measured by flow cytometry demonstrated a large variation along the circadian time scale for each 24-h profile, with a range of variation from 29% to 339% (mean $118.2 \pm 18.4\%$) from lowest to highest value, with the highest DNA synthetic activity between 7 a.m. and 4 p.m. This has been confirmed in cancer patients (75). Finally, some experimental tumors exhibit similar rhythmic susceptibilty patterns, while others seem to vary little during the circadian cycle (12, 13). Studies of human tumors have confirmed this (76–78). Two clinical trials have demonstrated an asynchrony in DNA synthesis between tumor tissue and normal tissue (14, 15). Klevecz et al. (15) looked at cell proliferation in the ascites fluid from 30 patients with ovarian cancer. A highly significant circadian rhythm in tumor cell DNA synthesis was found. Its peak (mid to late morning) was found to be nearly 12 h out of phase with the proliferation in benign mesothelial cells from the same sample. In another study, patients with non-Hodgkin's lymphoma underwent thin-needle aspiration of tumor masses every 4 h for a minimum of 24 h. The within-day variation in S-phase values observed in individual patients ranged from 21% to 353%, and the majority of peak values were found late in the evening or during the night (14). This peak is 12 h out of phase with the circadian variation in S-phase in normal bone marrow (10).

Using short half life chemotherapeutic agents, such as the fluoropyrimidines, on an infusion schedule may have benefits both with regard to better activity and reduced toxicity (79). Infusion chemotherapy might therefore compliment the strategy of optimally timing chemotherapy. The availability of portable infusion pumps capable of delivering single or multiple drugs, each with their optimal circadian scheduling, has made the clinical application and testing of these principles possible (80). Clinical benefit using this scheduling strategy depends to some extent upon a certain cytokinetic and metabolic asynchrony between the circadian susceptibility patterns of the normal tissues at risk for drug toxicity and the tumor. If this asynchrony exists (14, 15), then at the time when the normal tissues are less vulnerable to the toxic effects of a drug, the tumor may not be protected to the same extent. This would allow more dose intensive treatment to be given without increased toxicity, thus improving the therapeutic index. A similar argument can be made for the toxic biological response modifiers (IL-2, tumor necrosis factor, interferon), whereas those that are nontoxic (erythropoietin, G-CSF) might achieve a better therapeutic effect with a lower and less costly dose if given at the optimal time. Below the data for fluoropyrimidines are reviewed in some detail. The table summarizes all other available clinical data.

Fluoropyrimidines—Clinical trials of circadian delivery

Fluorodeoxyuridine (FUDR). Based on experimental data (20, 81, 82) von Roemeling & Hrushesky used a 14-day continuous infusion of FUDR in a series of clinical trials. To achieve a variable rate infusion pattern, the daily drug dose was divided into four portions of 68%, 15%, 2% and 15%. Each portion was infused over a 6-h span with the peak of the infusion from 3 p.m. to 9 p.m. Compared to a flat infusion the variable rate infusion was found to give substantially less toxicity than a constant infusion of the same dose. The mean dose intensity achieved on a variable rate infusion (0.645 ± 0.1 mg/kg/week) was 45% higher than the maximum tolerated dose intensity on a flat infusion schedule (0.446 ± 0.1 mg/kg/week). This FUDR schedule was found to be active in 68 consecutive patients with metastatic renal cell cancer, with 4 complete responses and 7 partial responses to give a $19.6 \pm 5.1\%$ (95% confidence limits) objective response rate (19). Other researchers have confirmed this (83–85). A prospective randomized multicentre trial is ongoing, comparing a 14-day circadian infusion of FUDR to a 14-day flat FUDR infusion, in patients with metastatic renal cell cancer (86). To date this study has documented less toxicity and more dose intensity on the circadian arm. In 50 patients with liver metastases from colorectal cancer receiving FUDR as intrahepatic infusion, toxicity in the form of cholestasis and jaundice was several fold less frequent, less severe, and occurred later, when circadian infusion was employed as compared to a flat infusion. Patients receiving the circadian modified infusion tolerated a 70% higher average dose intensity. Response rates in the two groups were similar (21). Focan et al. (87) have reported preliminary data from a randomized study of intravenous 5-FU and intrahepatic FUDR in 38 previously untreated patients with liver metastases from colorectal cancer. Patients in arm A received a flat infusion, while patients in arm B received a circadian infusion with peaks for 5-FU at 4 a.m. and for FUDR at 4 p.m. Stomatitis was dose limiting. No hepatic toxicity was noted. After the third course toxicity (alopecia, neutropenia and skin) became lower in arm B in spite of a higher dose intensity. More courses could be delivered in arm B. The response rate was similar in both arms (50–60%). The median survival was superior in arm B (40+ months vs. 19.6 months in arm A). More recent animal studies (88) indicate that the optimal systemic FUDR infusion should most likely peak 4–6 h later than was originally extrapolated from bolus studies. Even more benefit might be expected in future clinical studies using FUDR infusion, if this is taken into account. Ongoing clinical studies are using this information in the hope of further improving the therapeutic index of continuous infusion FUDR.

5-fluorouracil (5-FU). Murine data investigating 5-FU induced lethal toxicity (89, 90) and human pharmacology data for 5-FU (6) were the basis for subsequent clinical

Table

Clinical trials using circadian timing in cancer therapy

Trial type	Drugs	No. pts.	Disease	Schedule/ Times tested	Results	Refs.
Phase-I/II/III	FUDR	36	Advanced cancer	14 day flat infustion v. circ. infusion, peak 3–9 pm	Circ. infus. less toxic, allowing more DI	(20)
Phase-II	FUDR	68	Renal cell	14 day circadian infusion, peak 3–9 pm	CR + PR = 20%	(19)
Phase-II	FUDR	42	Renal cell	14 day circadian infusion, peak 3–9 pm	CR + PR = 14%	(84)
Phase-II	FUDR	42	Renal cell	14 day circadian infusion, peak 3–9 pm	PR = 10% (+ 10% in metastatic sites only)	(85)
Phase-II	FUDR	13	Renal cell	14 day circadian infusion, peak 3–9 pm	CR + PR = 62%	(83)
Phase-III	FUDR	82	Renal cell (trial ongoing)	14 day circadian infusion peak 8 pm–2 am vs. flat inf	Circadian arm: less toxic, allowed more dose delivery	(86)
Phase-III	FUDR i.h.	50	Colorectal	14 day flat infusion v. circ. infusion peak 3–9 pm	Same OR: 32–35% Circ: 70% more DI	(21)
Phase-III	5-FU i.v. FUDR i.h.	38	Colorectal	5 day flat infusion v. circ. inf 5-FU peak at 4 am FUDR peak at 4 pm	Same OR: 50–60% MS superior in circadian arm (40 + v. 19 months)	(87)
Phase-I	5-FU	35	Colorectal	5 day circ. infusion peak at 4 am	75% more DI compared to historical controls	(16)
Phase-I	5-FU LV.	14	Advanced cancer	14 day circ. infus. Peak at 3–4 am v 9–10 pm	Less toxicity with inf. peak at 9–10 pm	(18)
Phase-II	5-FU LV oxaliplatin	93	Colorectal	5 day circ. infus. ev. 3 weeks 5-FU + LV peak 4 am Oxaliplatin peak 4 pm	OR: 58% Responses unrelated to previous therapy	(17)
Phase-II	5-FU LV oxaliplatin	37	Colorectal (5-FU resistant)	5 day circ. infus. ev. 3 weeks 5-FU + LV peak 4 am Oxaliplatin peak 4 pm	OR: 43%	(91)
Phase-II	5-FU LV oxaliplatin	48	Colorectal (5-FU resistant)	4 day circ. infus. ev. 2 weeks 5-FU + LV peak 4 am Oxaliplatin peak 4 pm	OR: 38%	(92)
Phase-III	5-FU LV oxaliplatin.	186	Colorectal metastatic	5 day circ. infus. ev. 3 weeks 5-FU + LV peak 4 am Oxaliplatin peak 4 pm. vs, flat	Circadian arm: less toxic, allowed more dose delivery and better response rate	(28)
Phase-III	Doxorubicin Cisplatin	23	Ovary, bladder	A. Dox. 6 am. Cispl. 6 pm v. B. Dox. 6 pm. Cispl. 6 am	Less toxicity on schedule A	(113)
Phase-III	Doxorubicin Cisplatin	37	Ovary	A. Dox. 6 am. Cispl. 6 pm v. B. Dox. 6 pm. Cisp. 6 am	Less toxicity on schedule A Surv. A: 40% v. B: 11%, (n.s.)	(25)
Phase-II	Doxorubicin Cisplatin	25	Endo-metrium	Dox. 6 am. Cispl. 6 pm	CR 4/25, 16% PR 9/25, 36%	(114)
Randomized Phase-II	THP-Doxorub Cisplatin	31	Ovary	A. THP 6 am. Cispl. 4–8 pm B. THP 6 pm. Cisplat 4–8 pm	A OR: 73%. Less toxicity B. OR: 57%. Less DI possible	(22)
Phase-I	Oxaliplatin	23	Advanced cancer	5 day flat infusion v. circ. infusion, peak 4 pm	Circ. less toxicity allowing a 33% ↑ DI	(23)
Crossover Phase-I	Carboplatin	7	Ovary	Bolus 400 mg/m^2 at 6 am vs. 6 pm	Less thrombocytopenia at 6 pm	(115)
Randomized Phase-II	VP-16 Cisplatin	34	Metastatic cancer	VP-16 at 7 am or 7 pm Cisplatin at 6 pm	Less bone marrow toxicity with VP-16 at 7 am	(116)
Phase-III	VP-16 Cisplatin	124	Advanced lung cancer	Cisplatin at 6 pm VP-16 at 6 am. v. 6 pm	Less marrow tox. at 6 am Cisplatin related toxicity less with VP-16 at 6 pm DI and responses same	(117)
Retrospect review	6-MP MTX	118	ALL	Treatment in the morning v. in the evening	Survival advantage for the evening group	(118, 119)

Abbreviations: OR: Objective response rate. DI: Dose intensity. i.v.: Intravenous. i.h.: Intrahepatic. MS: Median survival. Circ.: Circadian schedule. LV: Leucovorin. 6-MP: 6-mercaptopurin. MTX: Methotrexate. ALL: Acute lymphocytic leucemia. n.s.: Not statistically significant

trials, giving the highest dose of 5-FU at 4 a.m. Lévi et al. (16, 17) used a 5-day circadian continuous infusion of 5-FU every three weeks in thirty patients with metastatic colorectal cancer. The delivery rate varied in a circadian manner and was highest at 4 a.m. and null from 6 to 10 p.m. An intrapatient dose escalation by 1 g/m^2/course was planned from 5 g/m^2/course (usual schedule) to 9 g/m^2/course according to toxicity criteria. The mean highest tolerated dose was 7.5 g/m^2/course, considerably higher than that recommended for a constant infusion of 5-FU.

In a phase-II trial in patients with metastatic colorectal cancer, 5-FU, leucovorin (LV) and oxaliplatin were infused continuously for 5 days every 3 weeks (17). Oxaliplatin (25 mg/m^2/day) was infused for 12 h with peak delivery at 4 p.m., and 5-FU (700 mg/m^2/day) and LV (300 mg/m^2/day) were infused concurrently for 12 h with peak delivery at 4 a.m. Fifty-four of the 93 patients had an objective response (58%; 95% confidence limits 48% to 68%) irrespective of previous chemotherapy. In a subsequent phase-II study this circadian schedule was used in 37 patients with fluoropyrimidine resistant colorectal cancer (91). After a mean number of 8 courses, a partial response rate was achieved in 16/37 patients (43%). This activity in fluoropyrimidine resistant colorectal cancer has been confirmed in a second phase-II study giving the same dose of these drugs over 4 days every 2 weeks (20% increased dose intensity). A 38% response rate was achieved in 48 patients (92). The timing of oxaliplatin was based on previous experimental (93) and clinical studies (23). This circadian regimen has now been compared to a flat delivery of the same drugs in a prospective phase-III clinical trial in 186 patients with metastatic colorectal cancer (28). Patients on the circadian arm has less toxicity with regard to both stomatitis (grade 3–4 in 15% vs. 75%, $p < 0.0001$), hand–foot syndrome ($p < 0.03$), neutropenia ($p < 0.03$) and neuropathy (15% vs. 29%, $p < 0.05$). In spite of less toxicity, the median dose intensity (mg/m^2/week) for 5-FU over 6 planned cycles was significantly higher on the circadian arm (1013 vs. 815, $p = 0.00001$). The objective response rate was also significantly better on the circadian arm (49.5% vs. 30%, $p = 0.007$).

A phase-I trial has been done to identify the optimal dose rate of delivery for admixtures of 5-FU and LV given for 14 days as a flat continuous infusion (94). The optimal dose rate for 5-FU and LV was found to be 200 mg/m^2/day and 55 mg/m^2/day respectively. At this dose no toxicity greater than grade-I was seen. At higher doses of 5-FU (250 mg/m^2/d) or LV (10 mg/m^2/day), stomatitis became dose limiting. Bjarnason et al. (18) determined the maximum tolerated dose (MTD: $\geq$ 50% of patients with $\geq$ grade-II toxicity) for 5-FU and LV, given as a continuous circadian infusion over 14 days, with 64% of the daily dose given over 7 h around 3–4 a.m. LV was first escalated by 5 mg/m^2/day to 20 mg/m^2/day, followed by escalation of 5-FU by 50 mg/m^2/day. Patients who developed $\geq$ grade-II toxicity had the peak of the infusion shifted from 3–4 a.m. to 9–10 p.m., to determine if this reduced toxicity. This timing corresponds to the time of least toxicity from 5-FU in more recent murine studies, testing non-lethal doses (95–97). Recent clinical studies of 5-FU pharmacokinetics (98), 5-FU metabolism (98, 99) and gut cytokinetics (11) also suggest that an evening infusion peak may be less toxic than an infusion peaking at 3–4 a.m. The MTD for an infusion peaking at 3–4 a.m. was reached at dose level 5 (5-FU 250 mg/m^2/day-LV 20 mg/m^2/day). In 6 patients developing $\geq$ grade-II toxicity the peak of the infusion was shifted to 9–10 p.m. Toxicity was reduced in all six and further dose escalation was possible in 3 patients. The MTD for an infusion peaking at 9–10 p.m. was reached at dose level 6 (5-FU 300 mg/m^2/day-LV 20 mg/m^2/day). Stomatitis and hand–foot syndrome were dose limiting. There was no bone marrow toxicity. The recommended dose for phase-II studies using this schedule is 5-FU 250 mg/m^2/day and LV 20 mg/m^2/day with the infusion peak at 9–10 p.m. This is a 300% and 25% higher dose for LV and 5-FU respectively than was suggested to be safe for a flat infusion. A phase-II study is ongoing in metastatic colorectal cancer.

Mechanism. The mechanisms for the time-dependent toxicity of fluoropyrimidines are only partially understood. More than 80% of an administered dose of 5-FU is rapidly catabolized in the liver and extrahepatic sites. Thus catabolism largely determines the availability of 5-FU for anabolism to its active nucleotide analogs. The activity of dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme for fluoropyrimidine catabolism, has been shown to be highly circadian time-dependent in the liver and bone marrow of rats and in the mouse liver (100). Harris et al. (101) used an isolated perfused rat liver model to study if the hepatic elimination rate of 5-FU and total 5-FU metabolites exhibited a similar circadian pattern as that observed for DPD activity in rat liver homogenates. A circadian variation was observed in the elimination rate of 5-FU and its catabolites. There was a reciprocal relationship between the elimination rate of 5-FU and 5-FU catabolites. The circadian variation in the plasma levels of 5-FU in rats during continuous infusion was prevented with cyano-2,6-dihydroxpyrimidine, a strong inhibitor of DPD (102). Harris et al. (98) and Tuchman et al. (99) have independently demonstrated a circadian variation of DPD activity in human mononuclear cells with peak values occurring at 10 p.m.–2 a.m. and at 1 a.m. respectively (Fig. 1). An inverse relationship between DPD activity in peripheral blood mononuclear cells and plasma 5-FU concentration was demonstrated by Harris et al. (98) in their study of cancer patients receiving a protracted continuous infusion of 5-FU. The importance of DPD activity in determining clinical toxicity from fluoropyrimidines is further suggested by the extreme toxicity seen in DPD deficient patients (103–105). The activity

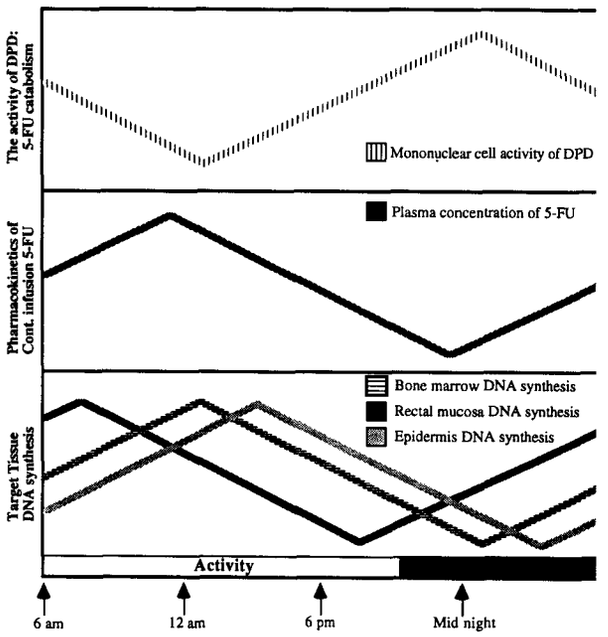

Fig. 1. Schematic representation of the available human data relevant to the pharmacokinetics and pharmacodynamics of 5-FU.

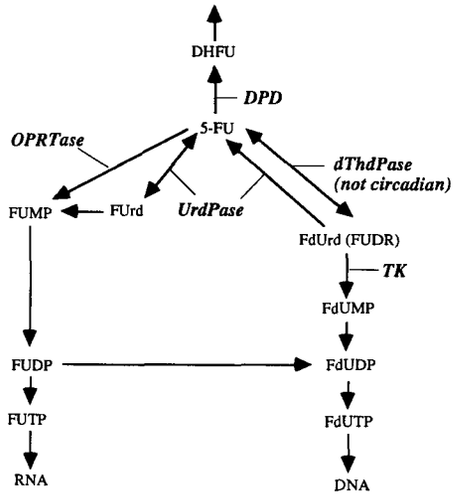

Fig. 2. Fluoropyrimidine metabolism. Enzymes that have been shown to have a circadian variation in activity and are described in text are shown in bold italic letters (dThdPase is not circadian). Other enzymes are not shown in this schematic diagram.

of three important enzymes of fluoropyrimidine anabolism, thymidine kinase (TK), orotate phosphoribosyltransferase (OPRTase) and uridine phosphorylase (UrdPase), has recently been shown to be circadian time-dependent in the rat bone marrow, -intestinal mucosa, -spleen and liver (106), as well as in mouse liver (100). In both of these studies, there was an inverse correlation between the activity of DPD and the activity of these anabolic enzymes such that when DPD (catabolism) was at its highest activity the anabolic enzyme activity was at a nadir and vice versa. Zhang et al. (106) found that survival rate was inversely correlated with TK activity (FUDR anabolism) and directly correlated with DPD activity (FUDR catabolism) in rats given FUDR at one of six circadian times. Studies, where inhibitors of UrdPase have been shown to increase the concentration and salvage of uridine and protect against host toxicity from both 5-FU and FUDR, attest to the importance of the circadian activity of UrdPase (107, 108).

El Kouni et al. (109) have indeed shown that the plasma concentration of uridine follows a circadian rhythm which is inverse of that for UrdPase activity in rodents. The peak activity of OPRTase in the study by Naguib et al. (100) concurs with the time reported for maximum incorporation of orotic acid into pyrimidine components of acid soluble extract, RNA and DNA in rat liver (110). No circadian variation was found in the activity of thymidine phosphorylase (dThdPase), another important fluoropyrimidine enzyme, in mouse liver (100), rat liver (111) or human mononuclear cells (112). The above experimental and clinical data support the contention that at the time of day when fluoropyrimidine catabolism is most active, anabolic activity is at its nadir and vice versa. This time-dependent variation in fluoropyrimidine metabolism could at least in part explain the apparent time dependent toxicity of fluoropyrimidines in both experimental and clinical trials (Fig. 2). In addition to the circadian pattern of activity of important biochemical pathways, circadian patterns of cytokinetic activity in normal tissues damaged by these drugs may be of equal importance in explaining the circadian pharmacodynamics. Both 5-FU and FUDR are most active against dividing cells during the process of DNA synthesis. Depending on the schedule of delivery, the gut, skin and bone marrow are the primary targets of fluoropyrimidine toxicity. Circadian rhythms have been demonstrated with regard to the amount of ongoing DNA synthesis, both in human skin (120), human bone marrow (10) and human colorectal mucosa (11) (Fig. 1).

Conclusion

Clinical trials to date have confirmed the experimental findings that optimal timing of chemotherapy can lead to decreased toxicity and allow delivery of more dose intensive therapy. One prospective trial has documented improved response rates using this scheduling method (28). The mechanisms responsible for this observation are only partly understood. These data have several potential implications for the conduct of both experimental and clinical studies. If all drugs were delivered at a predetermined time in phase-I and phase-II clinical trials, the heterogeneity commonly seen in both toxicity and achievable dose intensity might be reduced. If the ability of circadian chemotherapy to improve response rates is validated in further studies (28), phase-III studies may be comparing apples

and oranges to some extent if all agents in all arms are not delivered at the 'optimal time'. Circadian deliver of chemotherapy could complement other strategies for high dose therapy, since this scheduling reduces not only bone marrow toxicity but also other organ toxicities. Expermental studies indicate that the biological response modifiers may be even more time dependent, both with regard to toxicity and activity. This remains to be tested in clinical trials. Finally some common misconceptions about circadian delivery of chemotherapy should be addressed. No single time of day is best for every chemotherapy drug. Each drug has its own optimal time with regard to reduced toxicity and the ability to achieve a higher dose intensity. The timed delivery of drugs is not meant to make chemotherapy easy. This can be achieved simply by reducing the delivered dose. Circadian delivery of chemotherapy allows the delivery of a higher dose intensity without more toxicity. In the hope of achieving better anticancer effect, the dose of drugs should always be escalated for each patient until acceptable toxicity, as defined by the doctor and the patient, is generated.

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