The Role of Clock Genes in Pharmacology

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Abstract

The physiology of a wide variety of organisms is organized according to periodic environmental changes imposed by the earth's rotation. This way, a large number of physiological processes present diurnal rhythms regulated by an internal timing system called the circadian clock. As part of the rhythmicity in physiology, drug efficacy and toxicity can vary with time. Studies over the past four decades present diurnal oscillations in drug absorption, distribution, metabolism, and excretion. On the other hand, diurnal variations in the availability and sensitivity of drug targets have been correlated with time-dependent changes in drug effectiveness. In this review, we provide evidence supporting the regulation of drug kinetics and dynamics by the circadian clock. We also use the examples of hypertension and cancer to show current achievements and challenges in chronopharmacology.

INTRODUCTION

Diurnal: referring to a period of one day as a result of both the internal timing system and external environmental stimuli

Circadian: referring to a period of approximately one day as a result of the internal timing system independent of external stimuli

Mammalian physiology is organized so that it can sense and consolidate environmental cues such as light, temperature and—as shown recently (1)—magnetic fields to anticipate environmental changes that derive from the rotation of the earth. As a consequence of this environmental adaptation, mammals possess an internal clock and present diurnal rhythmicity in many physiological functions. The rhythms that derive from the internal timing system persist in the absence of environmental stimuli and are called circadian from the Latin words *circa* (approximately) and *dies* (day). Because of the broad influence of the circadian clock on mammalian physiology, it is expected that the clock also gates the response of the organism to drugs. During evolution the development of the circadian system coincided with the development of cell metabolism.

Numerous studies provide evidence for the mutual relationship between cell metabolism and the circadian clock. The circadian clock imposes a rhythm on many metabolic pathways, whereas transcription factors that sense nutrients and metabolites provide positive and negative feedback in the transcriptional circuit of the circadian clock. As part of this relationship, the circadian clock may influence drug metabolism in a variety of ways. Circadian variation in the activity of many gastrointestinal, hepatic, and renal processes could explain why the absorption, distribution, metabolism, and excretion of drugs change as a function of the time of drug administration. Thus, it is possible to predict temporal changes in the kinetics of drugs when the chronobiology of the pathways involved in the absorption, distribution, metabolism, and excretion of drugs is known. On the other hand, the plasma levels of drugs may vary with time even when drugs are administered through parenteral injections at different times of the day (2–4) or by continuous intravenous infusion (5). These examples emphasize the existence of diurnal variations not only at the level of enteral drug absorption but also in drug distribution, metabolism, and elimination.

The aim of chronopharmacology is to utilize rhythms of physiology to try to synchronize concentration and dosing of medications to increase their efficacy and safety. The clinical use of chronopharmacology is attracting more attention as the role of the clock in regulating both pharmacodynamics and pharmacokinetics is increasingly appreciated. So far, only observational studies have been performed to assess the effect of timing of drug administration in their efficacy and toxicity. Even when the importance of the time of administration has been well established—as, for example, in the case of nonsteroidal anti-inflammatory drugs—the impact on practical therapeutics has been minimal. The refinement of our understanding of diverse and pleiotropic impacts of the clock on physiology and disease will lead to a renewed approach to chronopharmacology. A few such studies in animal models where discrete elements of the molecular clock have been genetically manipulated have already been conducted.

DIURNAL AND CIRCADIAN CHANGES IN DRUG KINETICS

Rhythms in Drug Absorption

The absorption of a number of widely used drugs such as nitrates (6), benzodiazepines (7, 8), calcium channel blockers (9), acetaminophen (paracetamol) (10), and antidepressants (11) was found to be more rapid after oral administration in the morning compared to in the evening. For all the above-mentioned drugs, the rate of absorption was increased, the maximal plasma serum concentration (C_{max}) was greater, and the time after administration needed to obtain maximal plasma levels (t_{max}) was shorter when the drugs were taken at the beginning of the day. The differences in drug absorption at different times of the day may be the result of diurnal changes in various aspects of physiology.

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Diurnal variation in gastric emptying time and blood flow in the gastrointestinal tract may result in time-dependent variation in drug absorption. Goo et al. (12) measured the gastric emptying time when an identical isotopic meal was given to 16 healthy volunteers at 8:00 a.m. and 8:00 p.m. The gastric emptying of solids measured 60 and 80 min after meal ingestion was, respectively, 25% and 18% faster on average at 8:00 a.m. than at 8:00 p.m. Diurnal variation was also found in the gastrointestinal motility in humans; motility in the daytime being double that in the evening or at nighttime (13). Increased gastric emptying and motility during the day compared to the night results in increased absorption of lipophilic drugs when they are administered in the morning compared to the evening. Diurnal changes in blood flow to the gastrointestinal tract can also contribute to the time-dependent variation in the absorption of drugs. The apparent blood flow in healthy male volunteers was determined from the kinetics of indocyanine green. The peak of estimated blood flow obtained at the beginning of the day was significantly higher than at three other times of day (14). Gastric acidity shows a diurnal rhythm, modified by buffering meals and nocturnal duodenal-gastric reflux, with the greatest rate of gastric acid secretion occurring in the evening and the smallest in the morning (15). Increased gastric acidity reduces the absorption of lipophilic drugs, and the diurnal variation in gastric acidity contributes to differences in drug absorption over different times of the day.

Exceptions to the increase in drug absorption after drug administration in the morning compared to the evening were found for dexamethasone (16), methotrexate (17), mercaptopurine (17, 18), and intramuscularly injected meperidine hydrochloride (pethidine) (19). Evening administration of these drugs resulted in higher drug plasma levels compared to morning administration. In the case of nortriptyline, morning and evening administration produced similar plasma levels of the drug (20).

Drug solubility and route of administration may influence the diurnal variability of drug absorption. Lipid-soluble drugs seem more likely to show temporal variations in pharmacokinetics than do water-soluble drugs. Langner & Lemmer (21) reported a clear time-dependent variation in the absorption of propranolol, a lipophilic drug: Peak plasma propranolol concentration was higher and t_{max} was shorter in the morning than in the evening. On the other hand, Shiga and colleagues (22) found no time-dependent variations in the absorption of atenolol, a more water-soluble β-adrenergic receptor antagonist, given orally to 13 hypertensive patients. In a random crossover study, Yoshiyama et al. (23) administered the same formulation of sodium valproate to eight male volunteers at 8:30 a.m. and 10:30 p.m. by the oral or rectal routes. t_{max} was shorter in the morning when the drug was administered orally, but such a temporal variation was absent after rectal administration.

Rhythms in Drug Distribution

Little is known about diurnal variation in the distribution of drugs to their site of action. Time-dependent variation in drug binding to plasma proteins is likely to influence the distribution of drugs that are highly protein bound and have a small volume of distribution. The binding capacity of the plasma corticosteroid-binding globulin, transcortin, for prednisolone varies with time in humans, with maximum binding occurring at midnight and minimum at 8:00 a.m. (24). Patel and colleagues (25) determined the binding of valproic acid to plasma proteins in samples collected from six healthy volunteers at 2-h intervals during the day. The free valproate ratio in plasma varied significantly with time and was maximal during early morning and minimal during late afternoon. Drug distribution is also dependent on the permeability of membranes to drugs. Bruguerolle & Jadot used the erythrocyte as a model to quantify the passage of drugs through membranes. Using lidocaine, they found that the erythrocyte to total plasma concentration ratio

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was 0.74 and 0.48 when the drug was administered in activity and resting periods, respectively (26).

Rhythms in Drug Metabolism

The liver is the major site of drug metabolism. Oxidation and conjugation to endogenous substrates are the two main reactions in drug metabolism, and both are described to change with time. The first study to report a temporal correlation between the metabolism and the effect of a drug was conducted by Nair & Casper (27). Injection of a barbiturate at different times of the day revealed an inverse relationship between the hexobarbital oxidase activity in the liver and the sleeping time produced by the drug.

The cytochrome P450 monooxygenase system. The cytochrome P450 monooxygenase system is the main system responsible for drug oxidation. Several early studies reported temporal variation in cytochrome P450 enzymes (28, 29). More recently, a functional genomics approach in mice provided evidence for the rhythmic expression of genes encoding cytochrome P450 enzymes that was under the control of an endogenous oscillator (30). In this study, the expression of more than 10,000 genes, assessed using high-density oligonucleotide arrays, was examined in mouse liver collected every 4 h for 2 days. The mice were kept in constant darkness and temperature during the experiment to exclude the possibility that these variables were driving observed rhythms in gene expression. The study revealed a circadian rhythm in the expression of genes encoding several members of cytochrome P450 and Alas1, encoding the rate-limiting enzyme in heme biosynthesis that is necessary for cytochrome P450 activity (30). Similar to the mRNA, protein levels of cytochrome P450 enzymes also describe circadian rhythms (31). More recently, P450 oxidoreductase mRNA was found to oscillate in a robust circadian rhythm (32). P450 oxidoreductase provides the electrons required for all cytochrome P450-mediated monooxygenase reactions (33). This suggests that the circadian variation of P450 oxidoreductase imposes a circadian rhythm in the activity of all cytochrome P450 enzymes (32).

Conjugation reactions. Acetaminophen (paracetamol) has been used as a model for the study of temporal variation in hepatic glucuronidation and sulfation reactions because these two pathways are mainly responsible for its elimination. The plasma half-life of acetaminophen was 15% longer at 6:00 a.m. than at 2:00 p.m., and the mean ratio of the glucuronide conjugate over unchanged acetaminophen excreted in the first 3.5-h urine sample varied between 5.2 at 6:00 a.m. and 7.8 at 2:00 p.m. (34). Another example of temporal variation in conjugation reactions comes from those that use glutathione as a substrate. Reduced glutathione forms an adduct with reactive intermediates of drugs produced by the cytochrome P450 monooxygenase system and promotes their detoxification. The hepatic concentrations of glutathione change with time, and this variation determines the diurnal changes in conjugation elimination of several drugs.

Rhythms in Drug Excretion

Diurnal rhythms have been described for glomerular filtration rate, effective renal plasma flow, tubular secretion, urine output, and urinary excretion of electrolytes and many endogenous substances (35). These rhythms may result in different excretion rates for drugs at different times of the day. The urinary excretion of albumin, transferrin, and immunoglobulin G is maximal around 4:00 p.m. and minimal around 3:00 a.m. (36). The differences in drug excretion rate at different times of the 24-h cycle described in healthy individuals are not related to day-night differences in

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activity because they persist during constant bed rest (37). It is more likely that diurnal variations in systemic blood pressure, the renin-angiotensin system, and renal blood flow are responsible for time-dependent changes in renal hemodynamics. However, to our knowledge, no studies have shown an effect of the diurnal variation of any of those parameters on drug excretion rate at different times of the day. Urinary pH is another important factor in the urinary excretion of drugs that shows diurnal variations. Passive reabsorption of drugs depends on urinary pH, because renal tubular cells are less permeable to the ionized form of weak acids, alkalis. Amphetamine is more strongly ionized at lower pH, and it is readily excreted in the urine. When the urinary pH is more basic, the non-ionized fraction is increased, and its urinary excretion is decreased markedly (38).

Studies in humans have indicated that urinary pH changes during the day; urinary pH is lower during the night and higher in the day (39). These temporal changes in urinary pH could explain why the excretion of salicylate (40) and sulfonamides (41) vary as a function of the time of day in humans. The pH difference over the 24-h span could also be important in explaining the renal toxicity of drugs such as the aminoglycosides (42). This results from their electrostatic binding to membrane acid phospholipids in proximal tubules (43). The interaction is pH dependent, and nephrotoxicity of gentamicin is greatest when rats are sleeping and least in the middle of the activity period. Thus, the risk of nephrotoxicity varied inversely with pH (44). Notably, rats—as well as mice—are nocturnal animals, so they are active during the night and they rest during the day, the opposite of humans. Diurnal changes in urinary pH were observed in fed rats but were absent when animals were fasted (39). Urinary pH was higher during food intake and decreased when the animals were not eating. Thus, food appears to influence the diurnal variation of urinary pH, which in turn modifies the binding or the excretion of drugs. This appears to be a limitation of observational studies. For this reason, observational studies cannot afford evidence conclusive of a role for the circadian system.

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DIURNAL AND CIRCADIAN CHANGES IN DRUG DYNAMICS

The interaction of a drug with its target is subject to time-dependent variation. Specific effects of drugs can generally be attributed to the interaction between the drug and its cellular target. The affinity of the drug for its target, the amount of the target present in a given tissue, and the baseline activity of the target system determine the effectiveness of the drug. Evidence for diurnal variation in pharmacodynamics comes from constant-rate drug infusion studies. The anticoagulant effect of constant-rate heparin infusion over 24 h in patients with deep vein thrombosis exhibits profound diurnal differences. The average anticoagulant effects of heparin varied twofold as a function of time of day in patients with deep vein thrombosis receiving a constant-rate infusion over 48 h (45, 46). The ability of constant-rate infusion of famotidine or ranitidine to raise intragastric pH varied threefold over 24 h despite a constant-rate infusion (47).

In this section we describe diurnal rhythms in two enzyme systems that constitute drug targets and are relevant to the cardiovascular system: the renin-angiotensin system and the nitric oxide–cyclic GMP system.

Renin-Angiotensin System

The renin-angiotensin system plays an important role in the homeostasis of blood pressure. The formation of the active agonist angiotensin II depends on the activity of two proteolytic enzymes: Renin cleaves angiotensinogen to form angiotensin I, and the angiotensin-converting enzyme (ACE) leads to conversion of the inactive angiotensin I into angiotensin II. Angiotensin II has

multiple effects on the vasculature, adrenal glands, kidneys, and brain, resulting in the regulation of systemic blood pressure and fluid homeostasis. Expression of components of the renin-angiotensin system exhibit considerable diurnal variation and therefore could potentially influence blood pressure circadian rhythms (48). Human plasma renin activity is low in the afternoon and increases during the night, as demonstrated in several clinical studies (49, 50). The diurnal variation in renin activity influences other factors, including the circulating levels of angiotensin I and angiotensin II (51).

On the basis of the variation in plasma renin activity, one could expect that inhibitors of the renin-angiotensin system would be more effective during the rest period. Indeed, an angiotensin-converting enzyme inhibitor and an angiotensin II AT_1-receptor antagonist were significantly more effective when administered in transgenic hypertensive TGR(mREN2)27 rats, carrying an additional mouse renin gene, during the daily rest period (52). The rest period is the time when both blood pressure (53) and plasma renin activity (54) are highest in this animal model of secondary hypertension. In patients with primary hypertension, an evening dosing of angiotensin-converting enzyme inhibitors was associated with higher efficiency in the reduction of blood pressure (55, 56). Similar findings have been reported for the effects of an angiotensin II AT_1-receptor antagonist (57).

In summary, rhythms in plasma renin activity, leading to diurnal variation in the formation of angiotensin peptides, have been documented in experimental animals as well as in humans and appear to be related to the clinical effects of inhibitors of the renin-angiotensin system.

Nitric Oxide–Cyclic GMP System

Endothelium-derived nitric oxide (NO) is an important regulator of vascular tone, and a loss of endogenous NO synthesis in hypertension has been implicated in the occurrence of cardiovascular complications associated with the disease. NO is synthesized in the endothelial cell by the enzyme NO synthase type III, also called eNOS. Once synthesized, NO leaves the endothelial cell by passive diffusion, enters adjacent vascular smooth-muscle cells and activates the cGMP-generating enzyme, soluble guanylyl cyclase. Studies in normotensive rats have shown that the urinary excretion of NO oxidation end products, a measure of NO synthesis, is higher during the night than in the daytime (58, 59). NO synthesis was reduced in 58-week-old rats and had no diurnal variation (58). Interestingly, young, spontaneously hypertensive rats showed a decrease in both the 24-h excretion of NO oxidation end products and the amplitude of its diurnal variation, resembling what happens in aged normotensive rats (58). These observations in studies with rats are very similar to the finding from a clinical study in normotensive and hypertensive humans. The urinary excretion of NO oxidation end products and its second messenger cGMP showed pronounced 24-h rhythmicity, with peak values at the end of the day and a trough in the second half of the night in healthy normotensive subjects. In contrast, the rhythmic pattern in NO oxidation end products and cGMP excretion was lost in hypertensive patients (60).

Rhythmic changes in the NO-cGMP pathway during the day have possible implications in the treatment of patients receiving drugs that are NO donors, such as organic nitrates. The acute application of glyceryl trinitrate (nitroglycerin) in patients with Prinzmetal's angina, characterized by coronary vasospasms in the absence of atherosclerotic lesions, led to a greater increase in the diameter of coronary arteries when the drug was given in the morning compared to when it was given in the afternoon (61). This finding correlates with observations that the urinary excretion of NO oxidation end products drops at night (60) and endothelium-dependent vasodilation by acetylcholine is smallest in the morning (62).

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THE MOLECULAR BASIS OF THE CIRCADIAN CLOCK

During the past decade, the molecular basis of the circadian clock has been identified in mammals. The circadian system is organized in a hierarchical manner with a master clock located at the suprachiasmatic nucleus (SCN), which lies above the optic chiasm at the base of the hypothalamus. The SCN receives information about the day-night cycle through photic input via a direct retinal innervation, the retinohypothalamic tract. The photic input arises from intrinsically photoreceptive retinal ganglion cells that express the photopigment melanopsin. Light-dependent glutamatergic activation of retinorecipient neurons by the retinohypothalamic tract initiates intracellular and intercellular cascades of gene expression in the SCN (63). In this way, brief exposures to light are sufficient to entrain the SCN clockwork to solar time, adjusting the oscillator to a precise 24-h cycle (64). Individual SCN neurons are competent biological clocks, but the sustainability and synchronization of the molecular oscillator depend on spontaneous electrical activity within the SCN and, specifically, neuropeptidergic signaling between SCN neurons (65–67). Neuropeptidergic signaling is responsible for interneuronal synchronization within the SCN (65). The intercellular communication of SCN neurons not only synchronizes rhythmicity of the SCN but is also required for the maintenance of the amplitude and precision of individual cellular oscillations (66).

A second synchronizer of the SCN clockwork is melatonin, a hormone secreted by the pineal gland (68, 69). A multisynaptic pathway extending from the medial hypothalamus to sympathetic afferents of the pineal gland drives nocturnal secretion of melatonin. The SCN projects directly to the paraventricular nucleus to activate neurons that send their axons to the intermediolateral column of the upper thoracic spinal cord, where they contact sympathetic preganglionic neurons that control pineal melatonin secretion (70). Through this indirect pathway, the SCN controls the sympathetic output to the pineal gland and generates the circadian cycle of melatonin secretion. Melatonin, in turn, phase-shifts circadian rhythms in the SCN by acting on MT_2 melatonin receptors expressed by SCN neurons, thus creating a reciprocal interaction between the SCN and the pineal gland (71). Melatonin's phase-altering effect is caused by its direct influence on the electrical and metabolic activity of the SCN (72).

The master oscillator located at the SCN communicates day-night cycle phase information to the rest of the body. Through neuronal and humoral signals, the SCN sends this information to peripheral circadian clocks that exist in almost all cells of the rest of the body and synchronize them to the same phase (73). At the same time, the clocks of the periphery are able to respond to other environmental cues such as temperature (74) and food intake (75) and alter their phase according to these cues.

The master and peripheral clocks share the same molecular makeup in mammals. Circadian oscillations are generated by a transcriptional and translational circuit consisting of positive and negative feedback loops (**Figure 1**). The basic helix-loop-helix transcription factors BMAL1, CLOCK, and NPAS2 form CLOCK:BMAL1 or NPAS2:BMAL1 heterodimers and drive transcription through E-boxes located within the promoters of various target genes. Among the target genes are period homolog (Per1–3), cryptochrome (Cry1–2), Rev-erbα, and retinoid-related orphan receptor alpha (Rorα). After a delay, the translated Per and Cry proteins heterodimerize, translocate to the nucleus, and repress CLOCK/NPAS2:BMAL1 heterodimers (76) (**Figure 1**). The Per and Cry heterodimers are progressively degraded, allowing the circuit to start again. This leads to a cycle in gene expression that takes approximately 24 hours to complete. The orphan nuclear receptor REV-ERBα, a heme sensor that regulates glucose homeostasis and energy metabolism, represses Bmal1 transcription through binding to a ROR element in the promoter of Bmal1 (77). On the other hand, RORα competes with REV-ERBα for the same ROR element and

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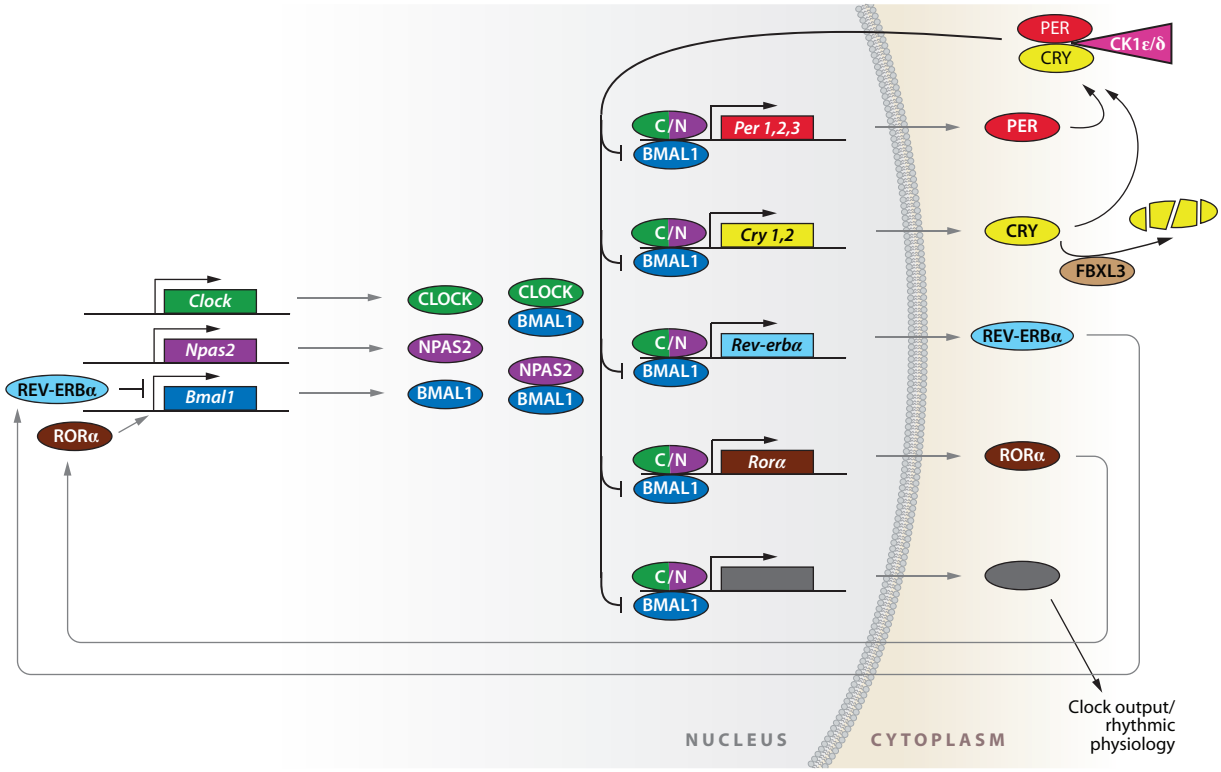

Figure 1

The mammalian circadian clock. The generation of circadian rhythms in gene expression is based on a transcriptional-translational feedback network. BMAL1 forms heterodimers with CLOCK and its paralog NPAS2. These heterodimers bind E-box *cis*-regulatory enhancer sequences and activate the transcription of genes such as Per1–3, Cry1 and 2, Rev-erbα, and Rorα. PER and CRY proteins heterodimerize, translocate to the nucleus, and interact with BMAL1:CLOCK/NPAS2 heterodimers to inhibit their transcriptional activity. After a certain period of time, the PER:CRY complex degrades and BMAL1:CLOCK/NPAS2 heterodimers start a new cycle of transcription. Additional feedback strengthens the robustness of the circadian clock. REV-ERBα and RORα compete for the same ROR response elements in the Bmal1 promoter. REV-ERBα inhibits, whereas RORα activates, the transcription of Bmal1. Posttranslational modification and degradation of clock proteins are crucial steps in circadian period. Phosphorylation of the PER:CRY heterodimer by CK1ε/δ is critical for the nuclear translocation of the complex. The FBXL3 targets CRY proteins for degradation. CK1ε/δ, casein kinase 1 epsilon/delta.

activates Bmal1 transcription (78). These additional feedback pathways provide further robustness to the circuit.

Accumulating evidence suggests a role for posttranslational modifications of the proteins of the circuit in the generation of circadian rhythms (79). Phosphorylation is critical for the transcriptional activity, stability, and cellular localization of the proteins in the feedback loop (80). Phosphorylation of PER and CRY proteins by casein kinase 1 delta/epsilon (CK1δ/ε) promotes the nuclear translocation of the PER:CRY complex (81). Recently, CK1δ/ε-dependent phosphorylation of PER2 was identified as the target of most of the pharmacologically active compounds found to lengthen the period of the circadian clock in an extensive screen in mouse and human cell lines (82). F-box protein FBXL3, a component of the SKP1/Cullin1/F-box ubiquitin ligase complex, targets CRY for proteolytic degradation—a critical event for the length of the circadian period (83–85). SUMOylation of Bmal1 affects its rhythmic expression (86), and dimerization

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of Bmal1 and Clock causes phosphorylation and directs nuclear accumulation of the complex (87).

The cellular reduction-oxidation (redox) state has also been implicated in the circadian regulation of gene expression. Rutter and colleagues (88) associated the ratio of reduced-to-oxidized nicotinamide adenine dinucleotide (NAD) with the DNA-binding activity of NPAS2:BMAL1 and CLOCK:BMAL1 in human neuroblastoma calls. According to their findings, the reduced form of NAD enhances the DNA binding of heterodimers as assessed by an electrophoretic mobility shift assay. More recently, Asher and colleagues (89) found that the NAD^+-dependent deacetylase SIRT1 binds PER2 in a circadian manner and promotes its deacetylation and degradation. Furthermore, silencing SIRT1 both by genetic deletion and by using siRNA reduced the amplitude of Bmal1 and Per2 expression oscillations but had no effect in their period length. In contrast to the findings of this study, Nakahata and colleagues showed that genetic ablation of SIRT1 causes an increase in the amplitude of Dbp and Per2 expression oscillations and found that SIRT1 interacts with CLOCK but not with PER2 (90). Two follow-up studies (91, 92) showed that the expression of Nampt, a gene encoding a rate-limiting enzyme in NAD^+ biosynthesis, is regulated by the CLOCK:BMAL1 heterodimer and oscillates with time, suggesting the existence of an additional feedback loop in the circadian circuit through NAD^+ and SIRT1. Future studies will likely shed light on the relative importance of the cellular redox state in the generation of circadian rhythms.

EVIDENCE FOR A ROLE OF THE CIRCADIAN CLOCK IN PHARMACOKINETICS

The identification of the circadian clock at the molecular level makes possible the transition from observational studies of drug efficacy and toxicity at different times of the day to cause-effect studies that provide a link between the circadian clock and drug metabolism. There are already reports showing a change in drug effectiveness or toxicity when the circadian clock is disturbed. Facilitating the performance of such studies, transgenic mice with disrupted biological rhythms are already available.

Albumin site d-binding protein (DBP), hepatocyte leukemia factor (HLF), and thyrotroph embryonic factor (TEF) are transcription factors of the PAR bZip family with gene expression directly regulated by the circadian clock (32). DBP, HLF, and TEF in turn regulate the expression of genes encoding drug-metabolizing enzymes and transporters, ALAS1 and POR, two enzymes required for the activity of all Class I monooxygenases, and CAR and PPARα, two well-known nuclear receptors involved in the control of drug metabolism. The circadian expression of Dbp, Hlf, and Tef drives a circadian rhythm in the expression of genes under their control and results in a circadian rhythm in multiple drug-metabolizing pathways. When Dbp, Hlf, and Tef are simultaneously knocked out, the response to drugs is altered. Administration of phenobarbital produces a low-level induction of Cyp2B10 in liver and small intestine of triple knockout mice throughout the 24-h cycle, whereas the induction in wild-type animals is daytime dependent. In agreement with this finding, barbiturate-induced sleep duration is constitutively high and dramatically increased in triple knockout animals in contrast to the time-of-injection-dependent sleep duration in wild-type mice.

A second, indirect association between drug metabolism and the circadian oscillation of drug-metabolizing enzymes showed a link between acetaminophen hepatotoxicity and CYP2E1 activity and hepatic glutathione levels (93). Both CYP2E1 activity and hepatic glutathione levels are under the control of the circadian clock and show a robust 24-h rhythm. The mortality caused by injection of acetaminophen into mice showed a diurnal variation that was related to circadian rhythms in CYP2E1 activity and hepatic glutathione levels. This dependency was shown by phase altering

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the rhythm of CYP2E1 and glutathione through restriction of food availability in the daytime. Food restriction reversed the phase in the ability of CYP2E1 and glutathione to metabolize acetaminophen and produced the same phase shift in the mortality rhythm (93).

As mentioned above, genome-wide analysis of gene expression revealed a circadian rhythm in the expression of many genes encoding proteins responsible for drug metabolism (30). Deletions of core clock genes validated the regulation of drug-metabolizing enzymes by the circadian clock in vivo and in vitro. Our group was able to identify RORα as a component of the circadian clock, being both a target gene for the BMAL1:CLOCK heterodimer and an activator of Bmal1 (78). A mouse with deletion of RORα showed the regulation in the expression of many genes encoding phase I and phase II proteins involved in the metabolism of lipids, steroids, and xenobiotics in mouse liver by this transcription factor (94). Among the genes regulated by RORα were several P450 (Cyp) genes, which catalyze oxidation and hydroxylation; Sult genes, which encode proteins that catalyze the sulfonation of xenobiotics; and glutathione transferases, which catalyze the conjugation of glutathione with a wide variety of xenobiotics, generally resulting in their detoxification and elimination. This finding provides evidence for the circadian regulation of drug metabolism and detoxification. In an in vitro study, knocking out Cry1 in a hepatic cell line reduced the amplitude of the rhythmic expression of Cyp2e1 (95). However, no studies yet link RORα or CRY1 with differential drug metabolism in different times of the day.

The best example of a study taking advantage of the current knowledge in the field of circadian biology to study drug toxicity is the study by Gorbacheva and colleagues (96). Wild-type mice showed variability in their tolerance to the anticancer drug cyclophosphamide (CY), which was dependent on the time of drug administration. Interestingly, the maximal and minimal sensitivity to the drug correlated with the trough and peak of CLOCK:BMAL1 transcriptional activity. This observation led Gorbacheva and colleagues to speculate that molecular determinants of sensitivity to CY could be directly regulated by CLOCK:BMAL1. To test this hypothesis, they compared the drug sensitivity of wild-type mice and mice with disturbed CLOCK:BMAL1 transcriptional activity. Bmal1 knockout mice have the minimal transcriptional activity of the complex because of the deficiency of transcriptional activators, whereas Cry1/Cry2 double knockout mice are deficient in circadian repression and express constantly high levels of CLOCK:BMAL1 transcriptional activity. Both Clock mutant and Bmal1 knockout mice showed increased sensitivity to the drug and loss of the circadian variability of the sensitivity to the drug. In contrast, animals with targeted disruption of both Cry genes were more resistant to the drug compared with their wild-type littermates. Again the time of the drug administration did not change the sensitivity of double Cry knockout mice to the drug.

Taken together, these observations suggest that the functional status of the major circadian CLOCK:BMAL1 transactivation complex determines sensitivity to the drug. The influence of the time of drug administration on toxicity in wild-type mice may reflect circadian variation in hepatic abundance or activity of drug-metabolizing enzymes or a circadian control of sensitivity of target cells to CY-induced cytotoxicity through modulation of the response to genotoxic stress. The time of drug administration had no effect on the rate of its metabolic transformation in both wild-type and Clock mutant mice, as the plasma levels of CY and its metabolites suggest. This was reproduced in vitro by co-culturing hepatocytes that metabolize CY from wild-type, Clock mutant, and Bmal1 knockout mice with test cells reporting the toxicity. There was no difference in the rate of CY metabolism. On the other hand, the reduction of circulating lymphocytes (the major target of CY-induced toxicity is the hematopoietic system manifested by severe lymphopenia and neutropenia, pronounced eosinophilia, and an altered lymphocyte/neutrophil ratio) was more severe in Clock mutant mice but less severe in double Cry knockouts compared to wild-type mice. This result indicates that the time-of-administration and genotype-related differences in response

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to CY-induced toxicity might reflect CLOCK:BMAL1-dependent modulation of lymphocyte survival and recovery rate. In agreement with the in vivo response, the reduction in lymphocytes was different for different administration times in wild-type mice, whereas Clock mutant mice showed the same reduction at both administration times. These data conclusively demonstrated that circadian control of drug response to CY in vivo is mediated through a CLOCK:BMAL1-dependent modulation of B cell survival and recovery.

CIRCADIAN VARIATION IN PHYSIOLOGY THAT DETERMINES THE TIMING OF DRUG ADMINISTRATION

Many chronic and acute medical conditions exhibit prominent circadian patterns of symptom manifestation and severity. Among them, cardiovascular events, such as angina pectoris (97), ventricular arrhythmia (98), acute myocardial infarction (99), sudden cardiac death (99), and thrombotic and hemorrhagic stroke (100), show strikingly higher frequency of appearance in the morning. The clinical severity of allergic rhinitis and bronchial asthma is most pronounced in early morning hours, and timed administration of medications against them has been reported (101). Numerous neurodegenerative and neuropsychiatric diseases have been associated with sleep disturbances, and melatonin has been suggested as a potential treatment (102). However, comparative evaluation of chronopharmacological strategies have been few—in blood pressure and some forms of cancer—and even then only with evaluation of impact on surrogate variables rather than clinical outcomes.

Circadian Variation in Blood Pressure

In humans with normal blood pressure and uncomplicated essential hypertension, blood pressure levels vary significantly with time over the 24-h daily cycle. Peak blood pressure levels occur during the mid morning and then decrease progressively throughout the remainder of the day to reach the lowest levels during nighttime sleep (103–105) (**Figure 2**). A slow but steady increase in blood pressure is then observed over the early morning hours before awakening, with an abrupt and steep increase during arousal and arising from overnight sleep. This morning blood pressure surge from low nighttime levels to higher daytime levels continues for 4 to 6 h after awakening, with a secondary dip early in the afternoon. In humans with a normal nocturnal decline in blood pressure, the sleep-time blood pressure mean is 10–20% lower than the daytime mean. In healthy young adults, the immediate morning rise of systolic blood pressure amounts to about 20–25 mm Hg, but in older adults the noncompliant vasculature can give rise to much greater 24-h variation in systolic and diastolic blood pressure, that is, 50 mm Hg or more. Activation of the sympathetic nervous system is an important mediator of the morning surge.

Disturbances of the circadian rhythm of autonomic nervous system activity and the neurohumoral factors that play a role in central and/or peripheral blood pressure regulation are clearly involved in the genesis of alterations in the typical 24-h blood pressure pattern. An imbalance of sympathetic versus parasympathetic activity is the major determinant of the alterations. This is true not only in various forms of neurogenic dysautonomias, but also in diabetes and chronic renal failure, where change in the circadian pattern is minimal (106) or absent (107), even before the clinical onset of autonomic neuropathy. Tonic activation of the sympathetic nervous system throughout the day and night is present in congestive heart failure. This seems to be the major determinant of observed changes in the blood pressure rhythm, as well as a presumed causal factor in congestive heart failure (108). A study of patients with varying degrees of progressive autonomic failure (affecting sympathetic and parasympathetic function) due to familial amyloid polyneuropathy displayed a progressive blunting of the circadian blood pressure rhythm with disease progression (109).

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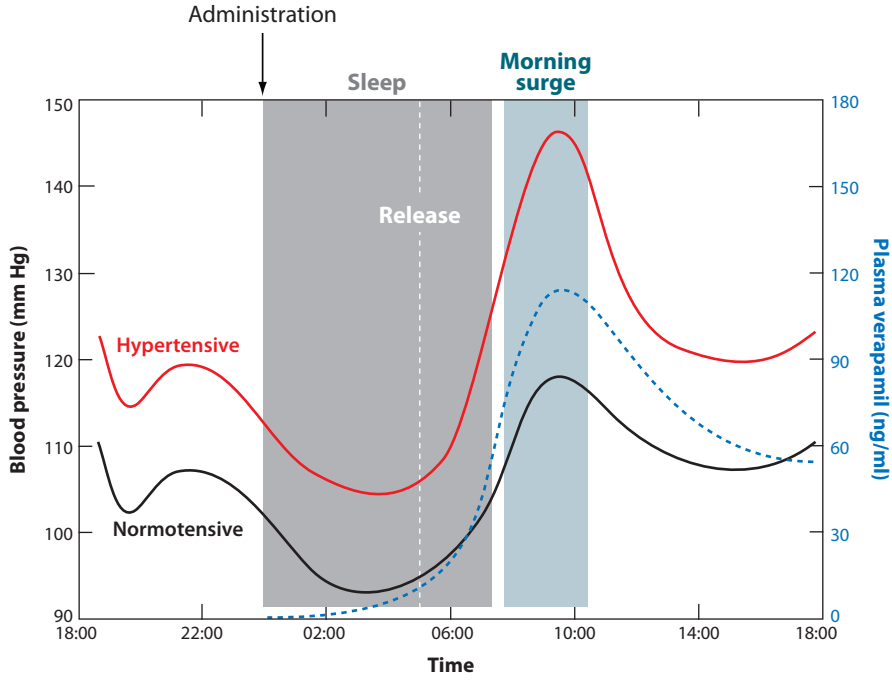

Figure 2

Diurnal variation of human blood pressure. The black line represents the mean blood pressure of a normotensive healthy human over time. Blood pressure decreases progressively starting at late afternoon to reach a trough value around 3:00 a.m. A slow increase in blood pressure is then observed over the early morning hours before awakening, followed by an abrupt and steep increase during arousal from overnight sleep. Blood pressure reaches a peak value during the mid morning and then decreases progressively throughout the remainder of the day. Hypertensive patients with an abnormally high rise of blood pressure during morning hours (mean blood pressure over time represented by the red line) may benefit from controlled-onset, extended-release medications. Bedtime administration of the delayed-delivery calcium channel blocker verapamil results in high plasma levels of the drug during the morning surge of blood pressure and sustained levels throughout the rest of the day (*dashed blue line*).

Although always assumed, evidence to support the direct role of the molecular clock in blood pressure regulation has been limited. An endogenous basis for the 24-h blood pressure variation, that is, a relationship between the circadian clock and the blood pressure rhythm, is suggested by studies in rodents showing that lesioning the SCN abolishes circadian rhythms of blood pressure and heart rate without affecting the sleep-wake and motor activity 24-h cycles (110). However, it is unknown how circadian information from the SCN is modulated and processed to regulate the 24-h rhythm of blood pressure and heart rate. The amplitude of the diurnal variation in blood pressure is increased in patients with hypertension, and the oscillation coincides with the temporal variability in their incidence of acute vascular events, such as myocardial infarction, sudden cardiac death, and stroke (111). Most normotensive individuals have a greater than 10% reduction in nighttime systolic blood pressure when compared with mean daytime values—dipping. The circadian pattern of blood pressure is maintained in hypertensive patients, although there is an upward shift to the blood pressure curve throughout the entire 24-h period compared with normotensive subjects, and the amplitude of the rhythm may be altered (112). However, some patients do not exhibit the nocturnal dip in blood pressure and are at increased risk of developing hypertension

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(113) and consequent end organ damage (114). For example, the absence of a normal drop in systolic blood pressure (known as nondippers) from day to night and the absolute nighttime diastolic blood pressure were both strong predictors of heart failure, stroke, and myocardial infarction, as well as sudden death in elderly patients with hypertension (114–117). More recently, Halberg and colleagues found that chronobiological analysis of human blood pressure recordings, interpreted in the light of reference values specified by gender and age, predicts actual and proxy outcomes when dipping fails (118).

Day to night differences in physical and mental activity are thought to be major determinants of blood pressure rhythmicity (119). Analysis of blood pressure rhythms in shift workers revealed an almost complete resynchronization within the first 24 h of the shift rotation. This may reflect variation in sympathetic activity, consistent with the correlation between diurnal variation in plasma catecholamines and blood pressure and heart rate. Sympathetic activity appears to integrate the major driving factors of temporal variability in blood pressure, but evidence also supports a role of the hypothalamic-pituitary-adrenal, hypothalamic-pituitary-thyroid, opioid, renin-angiotensin-aldosterone, and endothelial vasoregulatory systems, as well as other vasoactive peptides. Many hormones with established actions on the cardiovascular system, such as arginine vasopressin, vasoactive intestinal peptide, melatonin, somatotropin, insulin, steroids, serotonin, corticotropin-releasing factor, corticotropin, thyrotropin-releasing hormone, and endogenous opioids, show diurnal variations (120–126). Physical, mental, and pathologic stimuli, which may drive activation or inhibition of these neuroendocrine effectors of biologic rhythmicity, may also interfere with the temporal blood pressure structure.

However, the time-dependent responsiveness of cardiovascular tissues to such stimuli may be just as important. The recent use of telemetry systems suitable for small animals allows for recording of systolic blood pressure, diastolic blood pressure, heart rate, pulse pressure, and activity in unrestrained animals. The circadian pattern of blood pressure was examined in mouse models with disruption of the positive (BMAL1, CLOCK, and NPAS2) (127) or negative components (CRY1 and CRY2) of the oscillator (128). It was found that genes that subserve core functions in the molecular clock differentially regulate enzymes relevant to the synthesis and disposition of catecholamines. Both mean blood pressure and its variability over time are affected by disruption of the positive (BMAL1, CLOCK, and NPAS2) (127) or negative (CRY1 and CRY2) components of the oscillator (128). Plasma norepinephrine and epinephrine and the response to immobilization stress were also altered. Indeed, the absolute level of blood pressure and the baroreflex response to hypotension are differentially affected by deletion of BMAL1 or NPAS2 disruption on the one hand, versus deletion of both CRY1 and CRY2 on the other (128). Apparently, the circadian clock may influence the vascular response to stress indirectly by controlling the underlying rhythm of blood pressure on which asynchronous cues are imposed, but also directly by modulating pressor response, irrespective of timing. Both effects reflect the observed influence of the clock on sympathoadrenal function, which is activated in the integrated arousal response and many of its discrete elements, such as assumption of an upright posture, exercise, and emotional stress. Another study revealed that disruption of the baroreflex in rats results in loss of circadian variation in mean arterial pressure (129). We have previously reported that the baroreflex response, along with blood pressure, is subject to diurnal variation in humans (130).

Recently, we showed that deletion of Bmal1 in the vascular endothelium results in loss of the temporal pattern in susceptibility to thrombotic vascular occlusion secondary to vascular injury (131). The depression of endothelial Bmal1 reduced blood pressure during the active phase of the day and increased heart rate for both rest and active phases without changes in plasma catecholamines, nitric oxide biosynthesis, or fibrinolytic efficiency.

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Implications of the Circadian Rhythm of Blood Pressure in the Pharmacological Treatment of Hypertension

The treatment of hypertension has thus far mainly relied on lowering the level of (conventional clinic) blood pressure, without accounting for the normalization of the entire blood pressure pattern. As mentioned above, the extent of the nocturnal blood pressure decline predicts cardiovascular risk. Staessen and colleagues (116), using results from the Syst-Eur trial (in which nitrendipine was consistently dosed at bedtime), reported that nondippers experienced a greater incidence of stroke and myocardial infarction than people who had a normal dipping pattern. Even more relevant is the finding that dipper hypertensives had a relative hazard of cardiovascular mortality similar to that of nondipper normotensives (132). At the same time, the rate of blood pressure rise coincident with the commencement of diurnal activity has been identified as an independent predictor of the risk of morning stroke and acute coronary syndrome; it is also hypothesized to be a trigger for myocardial infarction at this time of day (99, 100, 133). So far, efforts to use the rhythm in blood pressure in the treatment of hypertension mostly compare the effectiveness of awakening versus bedtime dosing. The use of medications utilizing special drug-release technology has also been tested.

The controlled-onset, extended-release calcium channel blocker verapamil was the first special drug-delivery medication specifically designed for the therapy of hypertension. The drug-delivery technology of this tablet delays the release of verapamil for approximately 4–5 h following its recommended bedtime ingestion. Medication is released thereafter so the highest blood concentration is achieved in the morning around the time of awakening, with an elevated level sustained throughout diurnal activity (**Figure 2**). This type of medication was found to enhance the control of the morning blood pressure surge (134). The polymer-coated capsule of verapamil is a second special drug-delivery-based calcium channel blocker chronotherapy of hypertension. Release of verapamil from this capsule medication following recommended bedtime ingestion is delayed for approximately 4 h. Medication is then dispersed in an increasing amount so that peak blood concentration is achieved in the morning. This formulation achieves exaggerated reduction of blood pressure during the initial hours of diurnal activity as well as good control of blood pressure level throughout the 24-hour dosing interval (135). Graded-release, long-acting calcium channel blocker diltiazem and delayed release of the β-antagonist propranolol are other examples of special drug-delivery medication. Bedtime administration results in highest effectiveness for both (136, 137).

In secondary hypertension with an insufficient nightly fall in blood pressure (nondipping), evening dosing of calcium channel blockers has been shown not only to reduce the elevated blood pressure but also to normalize the pathological blood pressure profile from nondipping to dipping. Bedtime administration has proven to be more effective not only for calcium channel blockers but also for a number of other antihypertensive drugs such as angiotensin-converting enzyme inhibitors (138), α-adrenergic receptor antagonists (139), and angiotensin II receptor blockers (140). Surprisingly, low-dose (100 mg/day) aspirin, commonly used for cardioprotection, has a time-dependent hypotensive effect in humans. Thus, bedtime administration reduced blood pressure significantly, whereas morning administration failed to reduce blood pressure (141). Moreover, the reduction in nocturnal blood pressure with bedtime aspirin was particularly striking in nondippers. Clinical trials of low-dose aspirin for cardioprotection have not specified time of dosing. Pharmacological treatment of hypertension has to be individualized according to the 24-h pattern of blood pressure. Achievement of a normal blood pressure dipper pattern requires different strategies for patients with different blood pressure patterns. This might involve a unique time of the day of drug ingestion of conventional drug-delivered formulations and/or the application of advanced drug-delivery concepts and technology as required according to the blood

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pressure profile of each patient. Use of ambulatory blood pressure measurement is necessary for the identification of an individual's 24-h pattern and dipping pattern.

Chronopharmacology in the Treatment of Cancer

Circadian timing of drug delivery can play a significant role in anticancer therapeutic effectiveness and tolerability. By merely changing the time of day of drug delivery, variation in survival rate by more than 50% has been demonstrated in mice and rats following treatment with 30 or more anticancer drugs (summarized in Reference 142). Similar effects are observed in therapy of human cancer, with perhaps one of the most notable examples being the benefits of timed anticancer drug treatment of patients with metastatic colorectal carcinoma. Here we discuss the role of chronotherapy in cancer treatment, highlighting the growing evidence linking the circadian clock to both the cell cycle and cell proliferation, as well as possible mechanisms underlying chronomodulation of drug treatment.

Circadian Clocks and Cancer

There is increasing evidence linking chronic disruption of the sleep-wake cycle in humans with increased risk of certain cancers. The misalignment of circadian rhythms in physiology, endocrinology, metabolism, and behavioral rhythms with the external environment is common among humans that work night shifts or routinely travel across multiple time zones. An increased incidence of breast cancer have been observed among female flight attendants (143, 144). Similarly an increased risk of breast, colon, prostate, and endometrial cancers has been associated with rotating and/or permanent night shift work (145–151). These are merely associations with a lifestyle in which disruption of biorhythms is likely, but more persuasive data linking circadian disruptions and cancer have been found in rodents. Simulation of chronic jet lag, consisting of a 6-h phase advance or delay every week, results in increased mortality in aged mice (152). When these mice were inoculated with tumors, survival was worse than in mice kept in unshifted light-dark cycles (153). Furthermore, surgical ablation of the master clock in the SCN resulted in accelerated tumor growth and attenuated survival in mice (154).

The relationship between the rest-activity cycle and cancer prognosis in humans has been investigated in patients with metastatic colorectal cancer; patients with marked activity rhythms show a more favorable tumor response and survival rate and report a better quality of life (155). This and other animal studies suggest there is a link between the circadian clock and cell proliferation. Perhaps a normally functioning clock plays a role in preventing unchecked cellular proliferation. DNA synthesis and cellular proliferation can vary markedly in dividing mammalian cells, including human bone marrow, intestinal epithelium, and skin (156–162). Daily oscillation in the expression of several circadian clock genes associates with specific cell cycle phases in human oral mucosa (163), and several cell proliferation and cell cycle genes, such as the protooncogene c-myc, and cyclin B1, cdc2, wee1, Bub1, p55CDC, cyclin A2, and cyclin D1 (30, 164–166), are subject to diurnal oscillation.

Finally, several animals with a disrupted circadian clock show aberrant regulation of cell growth and proliferation and/or increased risk of developing tumors. For example, Clock mutant mice appear to have defects in proliferation and growth, and rhythmic expression of numerous genes that regulate cell growth are disrupted in these animals (167, 168). In addition, Per1 and Per2 may function as tumor suppressors. Mice deficient in Per2 function are cancer prone and have enlarged hyperplasia of the salivary gland by approximately one year of age as well as a higher number of colonic polyps than wild-type mice (164, 169). In some cell culture models, Per1 in addition to Per2

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functions like a tumor suppressor. Decreased Per levels led to increased growth rates and lower rates of apoptosis, and overexpression resulted in growth inhibition, cell cycle arrest, apoptosis, and decreased tumor formation size (170–174). Molecular disruptions and physical interactions offer some hints as to the role of these proteins in proliferation and response to DNA damage. Misregulation of the expression of c-myc, β-catenin, and several cyclins has been reported in Per1 and/or Per2 mutants, and Per1 was shown to physically associate with the DNA damage check point proteins ATM and Chk2 (164, 169, 170, 172). Finally, mice lacking an arrhythmic circadian clock owing to deletion of function cryptochrome genes have markedly impaired liver regeneration after partial hepatectomy, implicating the circadian clock in regulating the timing and efficiency of cell cycle events (165).

Chronomodulated Anticancer Therapy

There are numerous examples of the benefits of specifically timed daily delivery of chemotherapeutic agents. For example, children receiving 6-mercaptopurine and methotrexate for maintenance treatment of acute lymphoblastic leukemia showed about a twofold increase in disease-free survival if given treatment in the evening (175). Clinical studies for treatment of ovarian, renal, breast, and liver cancers showed that controlling circadian timing of dosage often leads to decreases in drug toxicity (175–181). We discuss the therapeutic advantages of timed drug delivery in some anticancer treatments in the context of the pharmacokinetics and pharmacodynamics that modulate such responses (summarized in **Figure 3**).

Probably one of the best-studied chronomodulated anticancer drug responses is treatment with 5-fluorouracil (5-FU). Several clinical studies have shown that when 5-FU is delivered at the same rate over the course of the day by continuous intravenous infusion, the mean plasma 5-FU levels fluctuate, with the highest levels late at night and lower levels at midday (4, 182–184). Understanding how 5-FU is metabolized led to an explanation of daily differences in its pharmacokinetics and toxicity. 5-FU functions to block DNA replication by inhibiting thymidylate synthase, a key enzyme in pyrimidine thymidine synthesis. Levels of thymidylate synthase activity rhythms in mouse bone marrow, intestinal mucosa, and oral mucosa vary up to twofold throughout the day (185). In addition, following continuous infusion of 5-FU in cancer patients, a daily fluctuation in the activity of dihydropyrimidine dehydrogenase (DPD), the initial and rate-limiting step in pyrimidine catabolism, was observed (5). DPD, the initial enzyme in the catabolism of 5-FU, exhibits daily oscillations in activity and mRNA expression in liver (186, 187). DPD peak activity in human cancer patients continually infused with 5-FU was out of phase with maximal plasma concentrations of 5-FU, with DPD activity peaking around midnight and 5-FU concentrations peaking around noon (5). Finally, survivability of mice given 5-FU changed according to the time of day of delivery, with maximal toxicity of 5-FU inversely correlated with DPD activity (188). Thus, the time-of-day differences in toxicity of 5-FU treatment are likely due to the diurnal regulation in enzymes involved in 5-FU metabolism, and this explains why toleration of 5-FU is highest when thymidylate synthase activity is low and DPD activity is highest.

The pharmacokinetics of 5-FU suggests that a chronomodulated delivery schedule would be most effective for cancer treatment, and numerous clinical trials have highlighted the benefits of chronotherapy of 5-FU, particularly in the treatment of colon cancer (142). Phase III clinical trials comparing a fixed time versus chronomodulated treatment of 5-FU, leucovorin, and oxaliplatin showed an increase in objective response from 32 to 53% with the use of timed drug administration (189). A larger phase III trial using the same drug delivery paradigm showed similar increases in efficacy with timed drug administration. In this study, an increase in objective response rate from ∼30 to ∼50%, as well as significant improvements in patient tolerability, were observed with

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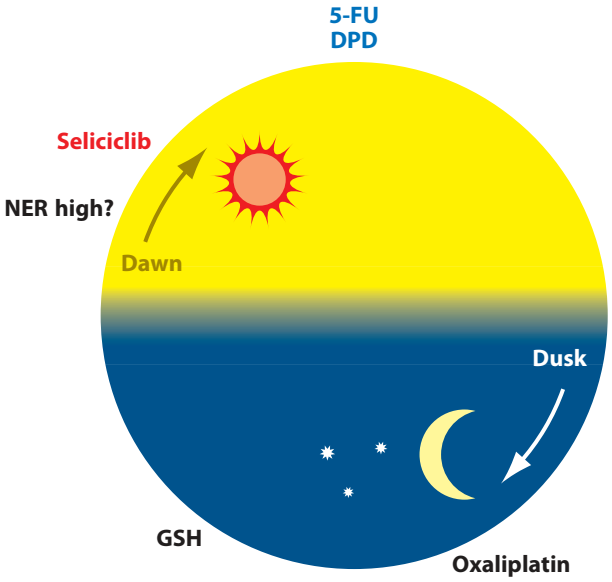

Figure 3

Chronotherapy of anticancer drugs in rodents. Time of day of most tolerated dosage is indicated for 5-fluorouracil (5-FU), oxaliplatin, and seliciclib. Dihydropyrimidine dehydrogenase (DPD), the initial enzyme in catabolism of 5-FU, exhibits daily oscillations in activity and mRNA expression in mouse liver, with peak levels during maximum 5-FU tolerability and efficacy. Seliciclib dosing in the morning is least toxic and most effective at shrinking tumor size in mice. Finally, oxaliplatin and other platinum-based anticancer drugs such as cisplatin show a time-of-day dependency in toxicity and efficacy. Reduced glutathione (GSH) levels are highest near the same time of day when cisplatin dosing is least toxic. Given that nucleotide excision repair (NER) targets the bulky lesions induced by cisplatin, repair activity may oscillate in target tissues to minimize drug toxicity to normal cells and increase efficacy of treatment in tumor cells.

chronomodulated drug delivery (190). The increased tolerability associated with chronotherapy in patients with metastatic colorectal cancer allowed for increases in dosing regimens of 5-FU as well as in the frequency of administration, which ultimately led to improvements in objective response rate and survival (191, 192).

Another example of a diurnal modulated drug effect is the chronotherapeutic effects of the cyclin-dependent kinase inhibitor seliciclib, which displays antitumor properties (193, 194). Mice inoculated with Glasgow osteosarcoma and then dosed with seliciclib at different times of day show a rhythm in drug tolerability and efficacy. Dosing in the morning was the least toxic and most effective in shrinking tumor size, whereas the most toxicity was observed around mid night during the middle of the mouse activity period (195). Treatment at this time also resulted in changes of the rhythmic expression patterns of several clock genes, as they changed from low amplitude or arrhythmic expression in untreated tumors to rhythmic expression patterns with phasing similar to their oscillations in the liver in seliciclib-treated mice. In addition, expression of wee1 changed from a low-amplitude oscillation to an arrhythmic pattern with a large increase in expression in seliciclib-treated mice (195). In the liver, wee1 kinase activity and expression are regulated by the circadian clock, thus making it a likely candidate for coordinating the cell cycle with the circadian clock (165). Therefore, seliciclib may modulate its chronotherapeutic effect through regulating wee1 levels, thus restoring the G2/M checkpoint and decreasing tumor growth. Another possible mode of clock–cell cycle interaction in these mice comes from the finding that seliciclib inhibits the function of CKI epsilon, which regulates period length in the circadian clock (79, 196).

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Toxicity and efficacy of the platinum-based anticancer drugs cisplatin and oxaliplatin fluctuate with a diurnal rhythm, and recent reports point toward a possible mechanism for these chronomodulated effects. Dosing of oxaliplatin during early to mid night led to decreased toxicity and tumor growth as well as an increase in life span in mice (197). Similar findings were observed in clinical studies on cancer patients, with chronomodulated drug delivery leading to better toleration of drug doses and improved objective response rates than reverse-phase or continuous drug delivery (179, 189). Reduced glutathione has been postulated as contributing to the detoxification of cisplatin because glutathione levels show daily fluctuations in liver, jejunum, and colon (35, 198, 199). Glutathione levels are highest during the night, which corresponds to the time of lowest cisplatin toxicity in mice (198, 199). Finally, cisplatin induces bulky DNA lesions that are targeted by the nucleotide excision repair pathway, and recently a circadian oscillation of this repair activity was observed in the mouse cortex (200). Although the phase of the nucleotide excision repair in other tissues is unknown, these findings suggest that timing of the highest DNA repair activity in healthy cells and the lowest activity in tumor cells may correspond with the time of day when the lowest drug toxicity and highest efficacy are observed.

Additional links between anticancer drug regulation and the clock have been suggested in studies with 5-FU. In mice with tumors, the time of day that 5-FU treatment associated with lowest toxicity, greatest antitumor effects, and best survival also correlated with maximal tumor nuclear BMAL1 protein accumulation and total WEE-1 protein levels (201). The relationships between clock protein accumulation and cell cycle components have not been extensively studied in cancer models, but the phase relationships between these systems may be important factors to consider in chronotherapeutics. For example, the presence or absence of a circadian clock, as well as the relationship between clock genes and cell cycle genes, may turn out to be important parameters to consider when assessing efficacy and tolerance of chronotherapeutics.

FUTURE DIRECTIONS IN CHRONOPHARMACOLOGY

Modern chronopharmacology is moving toward applying the current understanding of circadian rhythms to predict the circadian variability in drug effectiveness and toxicity. One of the major challenges in this effort is the identification of circadian oscillations at the protein level. Although we already have information about circadian changes in gene expression for a number of different tissues, the circadian variation of proteins is still largely unknown. Advances in quantitative proteomics technology will provide a proteome-wide analysis of circadian variations of proteins and better insight regarding the circadian rhythms in physiology. This will potentially enable prospective studies of drug effectiveness and toxicity designed to match the temporal variation in drug absorption, distribution, metabolism, and elimination. In comparison to observational studies performed so far, the advantage of the prospective approach comes from the distribution of the drug dose over time according to the diurnal variation of the relevant physiology. Apart from that, unraveling the circadian rhythms of proteins may enable drugs to act in an anticipatory way. Many of the nuclear receptors that regulate gene expression oscillate. Targeting drugs mediating their action through drug-induced gene activation against genes under circadian regulation may promote appropriate timing of action. The activation of Cyp2B10 by pentobarbital is such an example. Pentobarbital activation of Cyp2B10 is regulated by the constitutive androstane receptor (32) that oscillates in a circadian fashion. As a result, pentobarbital-induced sleep duration varies over time.

The appropriate timing of drug action may become feasible through progress in the technology of controlled drug release. A new class of controlled-release medication is based on hydrogels. Hydrogels can be sensitive to physical stimuli such as temperature, pH, and glucose concentration

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or to stimuli that can be externally implied such as electrical magnetic fields or ultrasound. Stimuli-sensitive hydrogels can be used to time drug release according to the rhythm in physiology. Finally, it is increasingly appreciated that coactivators and corepressors of the oscillatory transcriptome appear to exhibit some tissue specificity. This raises the possibility of being able to phase-shift drug sensitivity in a tissue-specific fashion that may both facilitate treatment paradigms and minimize toxicities in tissues other than those bearing the primary drug target.

SUMMARY POINTS

1. Drug absorption, distribution, metabolism, and excretion are different at different times of the daily cycle.
2. Drug targets—for example, in the renin-angiotensin and nitric oxide–cyclic GMP systems—show diurnal oscillations responsible for time-dependent drug effectiveness.
3. The circadian system generates rhythms by transcriptional-translational and posttranslational circuit-regulating gene expression.
4. Deletion of clock components reveals the regulation of drug effectiveness and toxicity by the circadian clock.
5. Circadian variations in physiology impose time-restrictions in drug administration for the therapy of diseases such as hypertension and cancer.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Yoshii T, Ahmad M, Helfrich-Forster C. 2009. Cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock. *PLoS Biol.* 7:e1000086
2. Choi JS, Kim CK, Lee BJ. 1999. Administration-time differences in the pharmacokinetics of gentamicin intravenously delivered to human beings. *Chronobiol. Int.* 16:821–29
3. Elting L, Bodey GP, Rosenbaum B, Fainstein V. 1990. Circadian variation in serum amikacin levels. *J. Clin. Pharmacol.* 30:798–801
4. Petit E, Milano G, Levi F, Thyss A, Bailleul F, Schneider M. 1988. Circadian rhythm-varying plasma concentration of 5-fluorouracil during a five-day continuous venous infusion at a constant rate in cancer patients. *Cancer Res.* 48:1676–79
5. Harris BE, Song R, Soong SJ, Diasio RB. 1990. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res.* 50:197–201
6. Scheidel B, Lemmer B. 1991. Chronopharmacology of oral nitrates in healthy subjects. *Chronobiol. Int.* 8:409–19
7. Nakano S, Watanabe H, Nagai K, Ogawa N. 1984. Circadian stage-dependent changes in diazepam kinetics. *Clin. Pharmacol. Ther.* 36:271–77
8. Muller FO, Van Dyk M, Hundt HK, Joubert AL, Luus HG, et al. 1987. Pharmacokinetics of temazepam after day-time and night-time oral administration. *Eur. J. Clin. Pharmacol.* 33:211–14

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

9. Lemmer B, Nold G, Behne S, Kaiser R. 1991. Chronopharmacokinetics and cardiovascular effects of nifedipine. *Chronobiol. Int.* 8:485–94
10. Kamali F, Fry JR, Bell GD. 1987. Temporal variations in paracetamol absorption and metabolism in man. *Xenobiotica* 17:635–41
11. Nakano S, Hollister LE. 1983. Chronopharmacology of amitriptyline. *Clin. Pharmacol. Ther.* 33:453–59
12. Goo RH, Moore JG, Greenberg E, Alazraki NP. 1987. Circadian variation in gastric emptying of meals in humans. *Gastroenterology* 93:515–18
13. Kumar D, Wingate D, Ruckebusch Y. 1986. Circadian variation in the propagation velocity of the migrating motor complex. *Gastroenterology* 91:926–30
14. Lemmer B, Nold G. 1991. Circadian changes in estimated hepatic blood flow in healthy subjects. *Br. J. Clin. Pharmacol.* 32:627–29
15. Moore JG, Englert E Jr. 1970. Circadian rhythm of gastric acid secretion in man. *Nature* 226:1261–62
16. Lamiable D, Vistelle R, Fay R, Millart H, Caron J, Choisy H. 1991. Chronopharmacokinetics of dexamethasone in young subjects. *Therapie* 46:405–7
17. Balis FM, Jeffries SL, Lange B, Murphy RF, Doherty KM, et al. 1989. Chronopharmacokinetics of oral methotrexate and 6-mercaptopurine: Is there diurnal variation in the disposition of antileukemic therapy? *Am. J. Pediatr. Hematol. Oncol.* 11:324–26
18. Langevin AM, Koren G, Soldin SJ, Greenberg M. 1987. Pharmacokinetic case for giving 6-mercaptopurine maintenance doses at night. *Lancet* 2:505–6
19. Ritschel WA, Bykadi G, Ford DJ, Bloomfield SS, Levy RC. 1983. Pilot study on disposition and pain relief after i.m. administration of meperidine during the day or night. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 21:218–23
20. Nakano S, Hollister LE. 1978. No circadian effect on nortriptyline kinetics in man. *Clin. Pharmacol. Ther.* 23:199–203
21. Langner B, Lemmer B. 1988. Circadian changes in the pharmacokinetics and cardiovascular effects of oral propranolol in healthy subjects. *Eur. J. Clin. Pharmacol.* 33:619–24
22. Shiga T, Fujimura A, Tateishi T, Ohashi K, Ebihara A. 1993. Differences of chronopharmacokinetic profiles between propranolol and atenolol in hypertensive subjects. *J. Clin. Pharmacol.* 33:756–61
23. Yoshiyama Y, Nakano S, Ogawa N. 1989. Chronopharmacokinetic study of valproic acid in man: comparison of oral and rectal administration. *J. Clin. Pharmacol.* 29:1048–52
24. Angeli A, Frajria R, De Paoli R, Fonzo D, Ceresa F. 1978. Diurnal variation of prednisolone binding to serum corticosteroid-binding globulin in man. *Clin. Pharmacol. Ther.* 23:47–53
25. Patel IH, Venkataramanan R, Levy RH, Viswanathan CT, Ojemann LM. 1982. Diurnal oscillations in plasma protein binding of valproic acid. *Epilepsia* 23:283–90
26. Bruguerolle B, Jadot G. 1983. Influence of the hour of administration of lidocaine on its intraerythrocytic passage in the rat. *Chronobiologia* 10:295–97
27. Nair V, Casper R. 1969. The influence of light on daily rhythm in hepatic drug metabolizing enzymes in rat. *Life Sci.* 8:1291–98
28. Belanger PM, Lalande M. 1988. Day-night variations in the activity and composition of the liver microsomal mixed-function oxidase of rat liver. *Annu. Rev. Chronopharmacol.* 5:219–22
29. Miyazaki Y, Imaoka S, Yatagai M, Motohashi Y, Kobayashi Y, Funae Y. 1990. Temporal variations in hepatic cytochrome P-450 isoenzymes in rats. *Annu. Rev. Chronopharmacol.* 7:149–52

Presents rhythmic expression of genes encoding enzymes of cytochrome P450.

30. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, et al. 2002. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109:307–20

31. Lavery DJ, Lopez-Molina L, Margueron R, Fleury-Olela F, Conquet F, et al. 1999. Circadian expression of the steroid 15 alpha-hydroxylase (Cyp2a4) and coumarin 7-hydroxylase (Cyp2a5) genes in mouse liver is regulated by the PAR leucine zipper transcription factor DBP. *Mol. Cell Biol.* 19:6488–99

Identifies a direct link between the circadian clock and drug metabolism.

32. Gachon F, Olela FF, Schaad O, Descombes P, Schibler U. 2006. The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. *Cell Metab.* 4:25–36

33. Gutierrez A, Grunau A, Paine M, Munro AW, Wolf CR, et al. 2003. Electron transfer in human cytochrome P450 reductase. *Biochem. Soc. Trans.* 31:497–501

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

34. Shively CA, Vesell ES. 1975. Temporal variations in acetaminophen and phenacetin half-life in man. *Clin. Pharmacol. Ther.* 18:413–24
35. Levi F, Schibler U. 2007. Circadian rhythms: mechanisms and therapeutic implications. *Annu. Rev. Pharmacol. Toxicol.* 47:593–628
36. Koopman MG, Krediet RT, Zuyderhoudt FJ, De Moor EA, Arisz L. 1985. A circadian rhythm of proteinuria in patients with a nephrotic syndrome. *Clin. Sci.* 69:395–401
37. Addis T, Barrett E, Poo LJ, Ureen HJ, Lippman RW. 1951. The relation between protein consumption and diurnal variations of the endogenous creatinine clearance in normal individuals. *J. Clin. Invest.* 30:206–9
38. Wilkinson GR, Beckett AH. 1968. Absorption metabolism and excretion of the ephedrines in man. I. The influence of urinary pH and urine volume output. *J. Pharmacol. Exp. Ther.* 162:139–47
39. Koike M, Norikura R, Mizojiri K, Sugeno K. 1984. Time-dependent elimination of cinoxacin in rats. *J. Pharmacol. Sci.* 73:1697–700
40. Markiewicz A, Semenowicz K. 1979. Time dependent changes in the pharmacokinetics of aspirin. *Int. J. Clin. Pharmacol. Biopharm.* 17:409–11
41. Dettli L, Spring P. 1967. Diurnal variations in the elimination rate of a sulfonamide in man. *Helv. Med. Acta* 33:291–306
42. Lin L, Grenier L, Theriault G, Gourde P, Yoshiyama Y, et al. 1994. Nephrotoxicity of low doses of tobramycin in rats: effect of the time of administration. *Life Sci.* 55:169–77
43. Laurent G, Carlier MB, Rollman B, Van Hoof F, Tulkens P. 1982. Mechanism of aminoglycoside-induced lysosomal phospholipidosis: in vitro and in vivo studies with gentamicin and amikacin. *Biochem. Pharmacol.* 31:3861–70
44. Beauchamp D, Collin P, Grenier L, LeBrun M, Couture M, et al. 1996. Effects of fasting on temporal variations in nephrotoxicity of gentamicin in rats. *Antimicrob. Agents Chemother.* 40:670–76
45. Decousus HA, Croze M, Levi FA, Jaubert JG, Perpoint BM, et al. 1985. Circadian changes in anticoagulant effect of heparin infused at a constant rate. *Br. Med. J.* 290:341–44
46. Hull RD, Raskob GE, Rosenbloom D, Lemaire J, Pineo GF, et al. 1992. Optimal therapeutic level of heparin therapy in patients with venous thrombosis. *Arch. Intern. Med.* 152:1589–95
47. Merki HS, Witzel L, Kaufman D, Kempf M, Neumann J, et al. 1988. Continuous intravenous infusions of famotidine maintain high intragastric pH in duodenal ulcer. *Gut* 29:453–57
48. Katz FH, Smith JA, Lock JP, Loeffel DE. 1979. Plasma vasopressin variation and renin activity in normal active humans. *Horm. Res.* 10:289–302
49. Portaluppi F, Bagni B, degli Uberti E, Montanari L, Cavallini R, et al. 1990. Circadian rhythms of atrial natriuretic peptide, renin, aldosterone, cortisol, blood pressure and heart rate in normal and hypertensive subjects. *J. Hypertens.* 8:85–95
50. Brandenberger G, Follenius M, Goichot B, Saini J, Spiegel K, et al. 1994. Twenty-four-hour profiles of plasma renin activity in relation to the sleep-wake cycle. *J. Hypertens.* 12:277–83
51. Veglio F, Pietrandrea R, Ossola M, Vignani A, Angeli A. 1987. Circadian rhythm of the angiotensin converting enzyme (ACE) activity in serum of healthy adult subjects. *Chronobiologia* 14:21–25
52. Schnecko A, Witte K, Lemmer B. 1995. Effects of the angiotensin II receptor antagonist losartan on 24-hour blood pressure profiles of primary and secondary hypertensive rats. *J. Cardiovasc. Pharmacol.* 26:214–21
53. Lemmer B, Mattes A, Bohm M, Ganten D. 1993. Circadian blood pressure variation in transgenic hypertensive rats. *Hypertension* 22:97–101
54. Lemmer B, Witte K, Schanzer A, Findeisen A. 2000. Circadian rhythms in the renin-angiotensin system and adrenal steroids may contribute to the inverse blood pressure rhythm in hypertensive TGR(mREN-2)27 rats. *Chronobiol. Int.* 17:645–58
55. Palatini P, Racioppa A, Raule G, Zaninotto M, Penzo M, Pessina AC. 1992. Effect of timing of administration on the plasma ACE inhibitory activity and the antihypertensive effect of quinapril. *Clin. Pharmacol. Ther.* 52:378–83
56. Witte K, Weisser K, Neubeck M, Mutschler E, Lehmann K, et al. 1993. Cardiovascular effects, pharmacokinetics, and converting enzyme inhibition of enalapril after morning versus evening administration. *Clin. Pharmacol. Ther.* 54:177–86

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

57. Pechere-Bertschi A, Nussberger J, Decosterd L, Armagnac C, Sissmann J, et al. 1998. Renal response to the angiotensin II receptor subtype 1 antagonist irbesartan versus enalapril in hypertensive patients. *J. Hypertens.* 16:385–93
58. Borgonio A, Witte K, Stahrenberg R, Lemmer B. 1999. Influence of circadian time, ageing, and hypertension on the urinary excretion of nitric oxide metabolites in rats. *Mech. Ageing Dev.* 111:23–37
59. Globig S, Witte K, Lemmer B. 1999. Urinary excretion of nitric oxide, cyclic GMP, and catecholamines during rest and activity period in transgenic hypertensive rats. *Chronobiol. Int.* 16:305–14
60. Bode-Boger SM, Boger RH, Kielstein JT, Loffler M, Schaffer J, Frolich JC. 2000. Role of endogenous nitric oxide in circadian blood pressure regulation in healthy humans and in patients with hypertension or atherosclerosis. *J. Investig. Med.* 48:125–32
61. Yasue H, Omote S, Takizawa A, Nagao M, Miwa K, Tanaka S. 1979. Circadian variation of exercise capacity in patients with Prinzmetal's variant angina: role of exercise-induced coronary arterial spasm. *Circulation* 59:938–48
62. Elherik K, Khan F, McLaren M, Kennedy G, Belch JJ. 2002. Circadian variation in vascular tone and endothelial cell function in normal males. *Clin. Sci.* 102:547–52
63. Hastings MH, Herzog ED. 2004. Clock genes, oscillators, and cellular networks in the suprachiasmatic nuclei. *J. Biol. Rhythms* 19:400–13
64. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. 2003. A phase response curve to single bright light pulses in human subjects. *J. Physiol.* 549:945–52
65. Maywood ES, Reddy AB, Wong GK, O'Neill JS, O'Brien JA, et al. 2006. Synchronization and maintenance of timekeeping in suprachiasmatic circadian clock cells by neuropeptidergic signaling. *Curr. Biol.* 16:599–605
66. Yamaguchi S, Isejima H, Matsuo T, Okura R, Yagita K, et al. 2003. Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302:1408–12
67. Harmar AJ, Marston HM, Shen S, Spratt C, West KM, et al. 2002. The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell* 109:497–508
68. Cassone VM, Chesworth MJ, Armstrong SM. 1986. Entrainment of rat circadian rhythms by daily injection of melatonin depends upon the hypothalamic suprachiasmatic nuclei. *Physiol. Behav.* 36:1111–21
69. Johnson RF, Moore RY, Morin LP. 1988. Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Res.* 460:297–313
70. Vrang N, Larsen PJ, Mikkelsen JD. 1995. Direct projection from the suprachiasmatic nucleus to hypophysiotrophic corticotropin-releasing factor immunoreactive cells in the paraventricular nucleus of the hypothalamus demonstrated by means of Phaseolus vulgaris-leucoagglutinin tract tracing. *Brain Res.* 684:61–69
71. Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, et al. 1997. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* 19:91–102
72. Pevet P, Bothorel B, Slotten H, Saboureau M. 2002. The chronobiotic properties of melatonin. *Cell Tissue Res.* 309:183–91
73. Schibler U, Sassone-Corsi P. 2002. A web of circadian pacemakers. *Cell* 111:919–22
74. Glaser FT, Stanewsky R. 2007. Synchronization of the *Drosophila* circadian clock by temperature cycles. *Cold Spring Harb. Symp. Quant. Biol.* 72:233–42
75. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. 2000. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 14:2950–61
76. Dunlap JC. 1999. Molecular bases for circadian clocks. *Cell* 96:271–90
77. Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, et al. 2002. The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110:251–60
78. Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, et al. 2004. A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* 43:527–37
79. Gallego M, Virshup DM. 2007. Post-translational modifications regulate the ticking of the circadian clock. *Nat. Rev. Mol. Cell Biol.* 8:139–48

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

80. Lee C, Etchegaray JP, Cagampang FR, Loudon AS, Reppert SM. 2001. Posttranslational mechanisms regulate the mammalian circadian clock. *Cell* 107:855–67
81. Akashi M, Tsuchiya Y, Yoshino T, Nishida E. 2002. Control of intracellular dynamics of mammalian period proteins by casein kinase I epsilon (CKIepsilon) and CKIdelta in cultured cells. *Mol. Cell Biol.* 22:1693–703
82. Isojima Y, Nakajima M, Ukai H, Fujishima H, Yamada RG, et al. 2009. CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc. Natl. Acad. Sci. USA* 106:15744–49
83. Busino L, Bassermann F, Maiolica A, Lee C, Nolan PM, et al. 2007. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. *Science* 316:900–4
84. Godinho SI, Maywood ES, Shaw L, Tucci V, Barnard AR, et al. 2007. The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. *Science* 316:897–900
85. Siepka SM, Yoo SH, Park J, Song W, Kumar V, et al. 2007. Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. *Cell* 129:1011–23
86. Cardone L, Hirayama J, Giordano F, Tamaru T, Palvimo JJ, Sassone-Corsi P. 2005. Circadian clock control by SUMOylation of BMAL1. *Science* 309:1390–94
87. Kondratov RV, Chernov MV, Kondratova AA, Gorbacheva VY, Gudkov AV, Antoch MP. 2003. BMAL1-dependent circadian oscillation of nuclear CLOCK: posttranslational events induced by dimerization of transcriptional activators of the mammalian clock system. *Genes Dev.* 17:1921–32
88. Rutter J, Reick M, Wu LC, McKnight SL. 2001. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293:510–14
89. Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, et al. 2008. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 134:317–28
90. Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, et al. 2008. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134:329–40
91. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. 2009. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. *Science* 324:654–57
92. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, et al. 2009. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. *Science* 324:651–54
93. Matsunaga N, Nakamura N, Yoneda N, Qin T, Terazono H, et al. 2004. Influence of feeding schedule on 24-h rhythm of hepatotoxicity induced by acetaminophen in mice. *J. Pharmacol. Exp. Ther.* 311:594–600
94. Kang HS, Angers M, Beak JY, Wu X, Gimble JM, et al. 2007. Gene expression profiling reveals a regulatory role for ROR alpha and ROR gamma in phase I and phase II metabolism. *Physiol. Genomics* 31:281–94
95. Matsunaga N, Ikeda M, Takiguchi T, Koyanagi S, Ohdo S. 2008. The molecular mechanism regulating 24-hour rhythm of CYP2E1 expression in the mouse liver. *Hepatology* 48:240–51
96. Gorbacheva VY, Kondratov RV, Zhang R, Cherukuri S, Gudkov AV, et al. 2005. Circadian sensitivity to the chemotherapeutic agent cyclophosphamide depends on the functional status of the CLOCK/BMAL1 transactivation complex. *Proc. Natl. Acad. Sci. USA* 102:3407–12
97. Rocco MB, Barry J, Campbell S, Nabel E, Cook EF, et al. 1987. Circadian variation of transient myocardial ischemia in patients with coronary artery disease. *Circulation* 75:395–400
98. Venditti FJ Jr, John RM, Hull M, Tofler GH, Shahian DM, Martin DT. 1996. Circadian variation in defibrillation energy requirements. *Circulation* 94:1607–12
99. Cohen MC, Rohtla KM, Lavery CE, Muller JE, Mittleman MA. 1997. Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. *Am. J. Cardiol.* 79:1512–16
100. Elliott WJ. 1998. Circadian variation in the timing of stroke onset: a meta-analysis. *Stroke* 29:992–96
101. Smolensky MH, Lemmer B, Reinberg AE. 2007. Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. *Adv. Drug Deliv. Rev.* 59:852–82
102. Pandi-Perumal SR, Srinivasan V, Maestroni GJ, Cardinali DP, Poeggeler B, Hardeland R. 2006. Melatonin: Nature's most versatile biological signal? *FEBS J.* 273:2813–38
103. Millar-Craig MW, Bishop CN, Raftery EB. 1978. Circadian variation of blood-pressure. *Lancet* 1:795–97
104. Mansoor GA, McCabe EJ, White WB. 1994. Long-term reproducibility of ambulatory blood pressure. *J. Hypertens.* 12:703–8

Reveals the regulation of sensitivity to cyclophosphamide by the core clock transactivation complex.

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

105. Weber MA. 2002. The 24-hour blood pressure pattern: Does it have implications for morbidity and mortality? *Am. J. Cardiol.* 89:27A–33A
106. Chau NP, Bauduceau B, Chanudet X, Larroque P, Gautier D. 1994. Ambulatory blood pressure in diabetic subjects. *Am. J. Hypertens.* 7:487–91
107. van de Borne P, Tielemans C, Collart F, Vanherweghem JL, Degaute JP. 1993. Twenty-four-hour blood pressure and heart rate patterns in chronic hemodialysis patients. *Am. J. Kidney Dis.* 22:419–25
108. Bell DS. 2003. Heart failure: the frequent, forgotten, and often fatal complication of diabetes. *Diabetes Care* 26:2433–41
109. Carvalho MJ, Van Den Meiracker AH, Boomsma F, Lima M, Freitas J, et al. 2000. Diurnal blood pressure variation in progressive autonomic failure. *Hypertension* 35:892–97
110. Janssen BJ, Tyssen CM, Duindam H, Rietveld WJ. 1994. Suprachiasmatic lesions eliminate 24-h blood pressure variability in rats. *Physiol. Behav.* 55:307–11
111. Muller JE. 1999. Circadian variation in cardiovascular events. *Am. J. Hypertens.* 12:35S–42S
112. Mancia G, Sega R, Milesi C, Cesana G, Zanchetti A. 1997. Blood-pressure control in the hypertensive population. *Lancet* 349:454–57
113. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Pede S, Porcellati C. 1998. Ambulatory pulse pressure: a potent predictor of total cardiovascular risk in hypertension. *Hypertension* 32:983–88
114. Verdecchia P, Schillaci G, Guerrieri M, Gatteschi C, Benemio G, et al. 1990. Circadian blood pressure changes and left ventricular hypertrophy in essential hypertension. *Circulation* 81:528–36
115. Rizzoni D, Muiesan ML, Montani G, Zulli R, Calebich S, Agabiti-Rosei E. 1992. Relationship between initial cardiovascular structural changes and daytime and nighttime blood pressure monitoring. *Am. J. Hypertens.* 5:180–86
116. Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, et al. 1999. Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. Systolic Hypertension in Europe Trial Investigators. *JAMA* 282:539–46
117. Ingelsson E, Bjorklund-Bodegard K, Lind L, Arnlov J, Sundstrom J. 2006. Diurnal blood pressure pattern and risk of congestive heart failure. *JAMA* 295:2859–66
118. Cornelissen G, Halberg F, Otsuka K, Singh RB, Chen CH. 2007. Chronobiology predicts actual and proxy outcomes when dipping fails. *Hypertension* 49:237–39
119. Clark LA, Denby L, Pregibon D, Harshfield GA, Pickering TG, et al. 1987. A quantitative analysis of the effects of activity and time of day on the diurnal variations of blood pressure. *J. Chronic. Dis.* 40:671–81
120. Landgraf R, Hacker R, Buhl H. 1982. Plasma vasopressin and oxytocin in response to exercise and during a day-night cycle in man. *Endokrinologie* 79:281–91
121. Quay WB. 1964. Circadian and estrous rhythms in pineal melatonin and 5-hydroxy indole-3-acetic acid. *Proc. Soc. Exp. Biol. Med.* 115:710–13
122. Lambert AE, Hoet JJ. 1966. Diurnal pattern of plasma insulin concentration in the human. *Diabetologia* 2:69–72
123. Liddle GW. 1966. Analysis of circadian rhythms in human adrenocortical secretory activity. *Arch. Intern. Med.* 117:739–43
124. Snyder SH, Zweig M, Axelrod J, Fischer JE. 1965. Control of the circadian rhythm in serotonin content of the rat pineal gland. *Proc. Natl. Acad. Sci. USA* 53:301–5
125. David-Nelson MA, Brodish A. 1969. Evidence for a diurnal rhythm of corticotropin-releasing factor (CRF) in the hypothalamus. *Endocrinology* 85:861–66
126. Clayton GW, Librik L, Gardner RL, Guillemin R. 1963. Studies on the circadian rhythm of pituitary adrenocorticotropic release in man. *J. Clin. Endocrinol. Metab.* 23:975–80
127. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA. 2007. Circadian variation of blood pressure and the vascular response to asynchronous stress. *Proc. Natl. Acad. Sci. USA* 104:3450–55
128. Masuki S, Todo T, Nakano Y, Okamura H, Nose H. 2005. Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking a biological clock. *J. Physiol.* 566:213–24
129. Makino M, Hayashi H, Takezawa H, Hirai M, Saito H, Ebihara S. 1997. Circadian rhythms of cardiovascular functions are modulated by the baroreflex and the autonomic nervous system in the rat. *Circulation* 96:1667–74

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

130. Hossmann V, Fitzgerald GA, Dollery CT. 1980. Circadian rhythm of baroreflex reactivity and adrenergic vascular response. *Cardiovasc. Res.* 14:125–29
131. Westgate EJ, Cheng Y, Reilly DF, Price TS, Walisser JA, et al. 2008. Genetic components of the circadian clock regulate thrombogenesis in vivo. *Circulation* 117:2087–95
132. Ohkubo T, Hozawa A, Yamaguchi J, Kikuya M, Ohmori K, et al. 2002. Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study. *J. Hypertens.* 20:2183–89
133. Kario K, Pickering TG, Umeda Y, Hoshide S, Hoshide Y, et al. 2003. Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives: a prospective study. *Circulation* 107:1401–6
134. White WB, Anders RJ, MacIntyre JM, Black HR, Sica DA. 1995. Nocturnal dosing of a novel delivery system of verapamil for systemic hypertension. Verapamil Study Group. *Am. J. Cardiol.* 76:375–80
135. Prisant LM, Devane JG, Butler J. 2000. A steady-state evaluation of the bioavailability of chronotherapeutic oral drug absorption system verapamil PM after nighttime dosing versus immediate-acting verapamil dosed every eight hours. *Am. J. Ther.* 7:345–51
136. Glasser SP, Neutel JM, Gana TJ, Albert KS. 2003. Efficacy and safety of a once daily graded-release diltiazem formulation in essential hypertension. *Am. J. Hypertens.* 16:51–58
137. Sica D, Frishman WH, Manowitz N. 2003. Pharmacokinetics of propranolol after single and multiple dosing with sustained release propranolol or propranolol CR (innopran XL), a new chronotherapeutic formulation. *Heart Dis.* 5:176–81
138. Morgan T, Anderson A, Jones E. 1997. The effect on 24 h blood pressure control of an angiotensin converting enzyme inhibitor (perindopril) administered in the morning or at night. *J. Hypertens.* 15:205–11
139. Panza JA, Epstein SE, Quyyumi AA. 1991. Circadian variation in vascular tone and its relation to alpha-sympathetic vasoconstrictor activity. *N. Engl. J. Med.* 325:986–90
140. Hermida RC, Calvo C, Ayala DE, Dominguez MJ, Covelo M, et al. 2003. Administration time-dependent effects of valsartan on ambulatory blood pressure in hypertensive subjects. *Hypertension* 42:283–90
141. Hermida RC, Ayala DE, Calvo C, Lopez JE, Fernandez JR, et al. 2003. Administration time-dependent effects of aspirin on blood pressure in untreated hypertensive patients. *Hypertension* 41:1259–67
142. Mormont MC, Levi F. 2003. Cancer chronotherapy: principles, applications, and perspectives. *Cancer* 97:155–69
143. Rafnsson V, Tulinius H, Jonasson JG, Hrafnkelsson J. 2001. Risk of breast cancer in female flight attendants: a population-based study (Iceland). *Cancer Causes Control* 12:95–101
144. Erren TC, Pape HG, Reiter RJ, Piekarski C. 2008. Chronodisruption and cancer. *Naturwissenschaften* 95:367–82
145. Davis S, Mirick DK, Stevens RG. 2001. Night shift work, light at night, and risk of breast cancer. *J. Natl. Cancer Inst.* 93:1557–62
146. Hansen J. 2001. Increased breast cancer risk among women who work predominantly at night. *Epidemiology* 12:74–77
147. Schernhammer ES, Kroenke CH, Laden F, Hankinson SE. 2006. Night work and risk of breast cancer. *Epidemiology* 17:108–11
148. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, et al. 2001. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Natl. Cancer Inst.* 93:1563–68
149. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, et al. 2003. Night-shift work and risk of colorectal cancer in the nurses' health study. *J. Natl. Cancer Inst.* 95:825–28
150. Kubo T, Ozasa K, Mikami K, Wakai K, Fujino Y, et al. 2006. Prospective cohort study of the risk of prostate cancer among rotating-shift workers: findings from the Japan collaborative cohort study. *Am. J. Epidemiol.* 164:549–55
151. Viswanathan AN, Hankinson SE, Schernhammer ES. 2007. Night shift work and the risk of endometrial cancer. *Cancer Res.* 67:10618–22
152. Davidson AJ, Sellix MT, Daniel J, Yamazaki S, Menaker M, Block GD. 2006. Chronic jet-lag increases mortality in aged mice. *Curr. Biol.* 16:R914–16

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

153. Filipski E, Delaunay F, King VM, Wu MW, Claustrat B, et al. 2004. Effects of chronic jet lag on tumor progression in mice. *Cancer Res.* 64:7879–85
154. Filipski E, King VM, Li X, Granda TG, Mormont MC, et al. 2002. Host circadian clock as a control point in tumor progression. *J. Natl. Cancer Inst.* 94:690–97
155. Mormont MC, Waterhouse J, Bleuzen P, Giacchetti S, Jami A, et al. 2000. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clin. Cancer Res.* 6:3038–45
156. Brown WR. 1991. A review and mathematical analysis of circadian rhythms in cell proliferation in mouse, rat, and human epidermis. *J. Invest. Dermatol.* 97:273–80
157. Buchi KN, Moore JG, Hrushesky WJ, Sothern RB, Rubin NH. 1991. Circadian rhythm of cellular proliferation in the human rectal mucosa. *Gastroenterology* 101:410–15
158. Garcia MN, Barbeito CG, Andrini LA, Badran AF. 2001. Circadian rhythm of DNA synthesis and mitotic activity in tongue keratinocytes. *Cell Biol. Int.* 25:179–83
159. Neal JV, Potten CS. 1981. Circadian rhythms in the epithelial cells and the pericryptal fibroblast sheath in three different sites in the murine intestinal tract. *Cell Tissue Kinet.* 14:581–87
160. Scheving LE, Pauly JE, von Mayersbach H, Dunn JD. 1974. The effect of continuous light or darkness on the rhythm of the mitotic index in the corneal epithelium of the rat. *Acta Anat. (Basel)* 88:411–23
161. Scheving LE, Tsai TH, Scheving LA. 1983. Chronobiology of the intestinal tract of the mouse. *Am. J. Anat.* 168:433–65
162. Smaaland R. 1996. Circadian rhythm of cell division. *Prog. Cell Cycle Res.* 2:241–66
163. Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, et al. 2001. Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. *Am. J. Pathol.* 158:1793–801
164. Fu L, Pelicano H, Liu J, Huang P, Lee C. 2002. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 111:41–50
165. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. 2003. Control mechanism of the circadian clock for timing of cell division in vivo. *Science* 302:255–59
166. Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, et al. 2002. Extensive and divergent circadian gene expression in liver and heart. *Nature* 417:78–83
167. Antoch MP, Gorbacheva VY, Vykhovanets O, Toshkov IA, Kondratov RV, et al. 2008. Disruption of the circadian clock due to the Clock mutation has discrete effects on aging and carcinogenesis. *Cell Cycle* 7:1197–204
168. Miller BH, McDearmon EL, Panda S, Hayes KR, Zhang J, et al. 2007. Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. *Proc. Natl. Acad. Sci. USA* 104:3342–47
169. Wood PA, Yang X, Taber A, Oh EY, Ansell C, et al. 2008. Period 2 mutation accelerates ApcMin/+tumorigenesis. *Mol. Cancer Res.* 6:1786–93
170. Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. 2005. The molecular clock mediates leptin-regulated bone formation. *Cell* 122:803–15
171. Gery S, Gombart AF, Yi WS, Koeffler C, Hofmann WK, Koeffler HP. 2005. Transcription profiling of C/EBP targets identifies Per2 as a gene implicated in myeloid leukemia. *Blood* 106:2827–36
172. Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. 2006. The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. *Mol. Cell* 22:375–82
173. Hua H, Wang Y, Wan C, Liu Y, Zhu B, et al. 2007. Inhibition of tumorigenesis by intratumoral delivery of the circadian gene mPer2 in C57BL/6 mice. *Cancer Gene Ther.* 14:815–18
174. Hua H, Wang Y, Wan C, Liu Y, Zhu B, et al. 2006. Circadian gene mPer2 overexpression induces cancer cell apoptosis. *Cancer Sci.* 97:589–96
175. Rivard GE, Infante-Rivard C, Hoyoux C, Champagne J. 1985. Maintenance chemotherapy for childhood acute lymphoblastic leukaemia: better in the evening. *Lancet* 2:1264–66
176. Depres-Brummer P, Berthault-Cvitkovic F, Levi F, Brienza S, Vannetzel JM, et al. 1995. Circadian rhythm-modulated (CRM) chemotherapy of metastatic breast cancer with mitoxantrone, 5-fluorouracil, and folinic acid: preliminary results of a phase I trial. *J. Infus. Chemother.* 5:144–47
177. Depres-Brummer P, Levi F, Di Palma M, Beliard A, Lebon P, et al. 1991. A phase I trial of 21-day continuous venous infusion of alpha-interferon at circadian rhythm modulated rate in cancer patients. *J. Immunother.* 10:440–47

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

178. Focan C, Denis B, Kreutz F, Focan-Henrard D, Levi F. 1995. Ambulatory chronotherapy with 5-fluorouracil, folinic acid, and carboplatin for advanced nonsmall cell lung cancer. A phase II feasibility trial. *J. Infus. Chemother.* 5:148–52
179. Hrushesky WJ. 1985. Circadian timing of cancer chemotherapy. *Science* 228:73–75
180. Hrushesky WJ, von Roemeling R, Lanning RM, Rabatin JT. 1990. Circadian-shaped infusions of floxuridine for progressive metastatic renal cell carcinoma. *J. Clin. Oncol.* 8:1504–13
181. Levi F, Benavides M, Chevelle C, Le Saunier F, Bailleul F, et al. 1990. Chemotherapy of advanced ovarian cancer with 4′-O-tetrahydropyranyl doxorubicin and cisplatin: a randomized phase II trial with an evaluation of circadian timing and dose-intensity. *J. Clin. Oncol.* 8:705–14
182. Bressolle F, Joulia JM, Pinguet F, Ychou M, Astre C, et al. 1999. Circadian rhythm of 5-fluorouracil population pharmacokinetics in patients with metastatic colorectal cancer. *Cancer Chemother. Pharmacol.* 44:295–302
183. Metzger G, Massari C, Etienne MC, Comisso M, Brienza S, et al. 1994. Spontaneous or imposed circadian changes in plasma concentrations of 5-fluorouracil coadministered with folinic acid and oxaliplatin: relationship with mucosal toxicity in patients with cancer. *Clin. Pharmacol. Ther.* 56:190–201
184. Takimoto CH, Yee LK, Venzon DJ, Schuler B, Grollman F, et al. 1999. High inter- and intrapatient variation in 5-fluorouracil plasma concentrations during a prolonged drug infusion. *Clin. Cancer Res.* 5:1347–52
185. Lincoln DW 2nd, Hrushesky WJ, Wood PA. 2000. Circadian organization of thymidylate synthase activity in normal tissues: a possible basis for 5-fluorouracil chronotherapeutic advantage. *Int. J. Cancer* 88:479–85
186. Daher GC, Zhang RW, Soong SJ, Diasio RB. 1991. Circadian variation of fluoropyrimidine catabolic enzymes in rat liver: possible relevance to 5-fluorodeoxyuridine chemotherapy. *Drug Metab. Dispos.* 19:285–87
187. Porsin B, Formento JL, Filipski E, Etienne MC, Francoual M, et al. 2003. Dihydropyrimidine dehydrogenase circadian rhythm in mouse liver: comparison between enzyme activity and gene expression. *Eur. J. Cancer* 39:822–28
188. Zhang R, Lu Z, Liu T, Soong SJ, Diasio RB. 1993. Relationship between circadian-dependent toxicity of 5-fluorodeoxyuridine and circadian rhythms of pyrimidine enzymes: possible relevance to fluoropyrimidine chemotherapy. *Cancer Res.* 53:2816–22
189. Levi FA, Zidani R, Vannetzel JM, Perpoint B, Focan C, et al. 1994. Chronomodulated versus fixed-infusion-rate delivery of ambulatory chemotherapy with oxaliplatin, fluorouracil, and folinic acid (leucovorin) in patients with colorectal cancer metastases: a randomized multi-institutional trial. *J. Natl. Cancer Inst.* 86:1608–17
190. Levi F, Zidani R, Misset JL. 1997. Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. International Organization for Cancer Chronotherapy. *Lancet* 350:681–86
191. Bertheault-Cvitkovic F, Jami A, Ithzaki M, Brummer PD, Brienza S, et al. 1996. Biweekly intensified ambulatory chronomodulated chemotherapy with oxaliplatin, fluorouracil, and leucovorin in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 14:2950–58
192. Levi F, Zidani R, Brienza S, Dogliotti L, Perpoint B, et al. 1999. A multicenter evaluation of intensified, ambulatory, chronomodulated chemotherapy with oxaliplatin, 5-fluorouracil, and leucovorin as initial treatment of patients with metastatic colorectal carcinoma. International Organization for Cancer Chronotherapy. *Cancer* 85:2532–40
193. Blagden S, de Bono J. 2005. Drugging cell cycle kinases in cancer therapy. *Curr. Drug Targets* 6:325–35
194. McClue SJ, Blake D, Clarke R, Cowan A, Cummings L, et al. 2002. In vitro and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine). *Int. J. Cancer* 102:463–68
195. Iurisci I, Filipski E, Reinhardt J, Bach S, Gianella-Borradori A, et al. 2006. Improved tumor control through circadian clock induction by Seliciclib, a cyclin-dependent kinase inhibitor. *Cancer Res.* 66:10720–28
196. Meng QJ, Logunova L, Maywood ES, Gallego M, Lebiecki J, et al. 2008. Setting clock speed in mammals: the CK1 epsilon tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. *Neuron* 58:78–88

Annu. Rev. Pharmacol. Toxicol. 2010.50:187-214.

197. Granda TG, D'Attino RM, Filipski E, Vrignaud P, Garufi C, et al. 2002. Circadian optimisation of irinotecan and oxaliplatin efficacy in mice with Glasgow osteosarcoma. *Br. J. Cancer* 86:999–1005
198. Boughattas NA, Li XM, Filipski J, Lemaigre G, Filipski E, et al. 1996. Modulation of cisplatin chronotoxicity related to reduced glutathione in mice. *Hum. Exp. Toxicol.* 15:563–72
199. Li XM, Metzger G, Filipski E, Boughattas N, Lemaigre G, et al. 1997. Pharmacologic modulation of reduced glutathione circadian rhythms with buthionine sulfoximine: relationship with cisplatin toxicity in mice. *Toxicol. Appl. Pharmacol.* 143:281–90
200. Kang TH, Reardon JT, Kemp M, Sancar A. 2009. Circadian oscillation of nucleotide excision repair in mammalian brain. *Proc. Natl. Acad. Sci. USA* 106:2864–67
201. Wood PA, Du-Quiton J, You S, Hrushesky WJ. 2006. Circadian clock coordinates cancer cell cycle progression, thymidylate synthase, and 5-fluorouracil therapeutic index. *Mol. Cancer Ther.* 5:2023–33

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