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CHRONOPHARMACOLOGY; PRINCIPLES AND APPLICATIONS

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ABSTRACT

Biological clocks and circadian rhythm Biological rhythms are innately determined rhythmic biological process or function. Circadian rhythms These are particularly important in medicine. Physiological day is about 25 hours where the clock is reset daily by the environment night day social schedules. Daily oscillations in abundance of proteins necessary for either drug absorption or metabolism result in circadian pharmacokinetics; and oscillations in the physiological systems targeted by these drugs result in circadian pharmacodynamics. These clocks are present in most cells of the body, but organized in hierarchical fashion. Interestingly, some aspects of physiology and behavior are controlled directly via a "master clock" in the suprachiasmatic nuclei of the hypothalamus, while others are controlled by "slave" oscillators in separate brain regions or body tissues. Recent research shows that these clocks can respond to different cues, and thereby show different phase relationships. Therefore, full prediction of chronopharmacology in pathological contexts will likely require a systems biology approach considering "chrono-interactions" among different clock-regulated systems.

KEYWORDS: Chronopharmacology, suprachiasmatic nucleus, hypothalamic-pituitary-adrenal axis, serotonergic system.

INTRODUCTION

Biological rhythms are innately determined rhythmic biological process or function and self-sustaining oscillation with the duration of time between successive repetitions (i.e., the period) being rather nonvarying under normal conditions. Rhythms affecting our body are ultradian cycles shorter than a day e.g. msec. for a neuron to fire; Circadian-Circa- about a

day, lasting for about 24 hours, e.g., sleep and wake cycles; Infradian- cycles longer than 24 hours e.g. menstrual cycle. Seasonal-like seasonal affective disorder causing depression in people during the short days of winter. While 24-hour clock times and sleep/wake rhythms frequently overlap with the internal clock, they do not always match the circadian rhythm.

There are a variety of methods to ascertain the timing of biological clocks.

- Melatonin provides the most reliable and consistent measure of the circadian pattern and can be measured in the plasma, saliva, or urine.
- Because secretion of the hormone is acutely suppressed by light exposure, the measurement of the time of onset of the daily melatonin rise during low-light exposure is a more reliable measure of the circadian phase.
- The dim-light melatonin onset (DLMO) has been used to assess alterations of circadian phase in a variety of diseases.
- Other markers, such as core body temperature, and cortisol may also serve as biomarkers for circadian rhythms.

Biologic rhythms are endogenous nature of circadian. Lack of external synchronizers leads to free running rhythms. The period of free-running rhythms is longer or shorter than 24 hours and is characteristic for each species. Our internal clocks are genetically determined. An internal biological clock is located in mammals, in the suprachiasmatic nucleus of the hypothalamus (SCN), delivering its message of time throughout the body. It is responsible for circadian rhythms and annual / seasonal rhythms. The SCN uses its connections with the autonomic nervous system for spreading its time-of-day message, either by setting the sensitivity of endocrine glands i.e., thyroid, adrenal, ovary) or by directly controlling an endocrine output of pineal gland i.e., melatonin. Mechanism of Chronopharmacology The basic unit of circadian timekeeping is the cell. Even in very complex organisms, most cells contain autonomous circuitry for circadian oscillations. Generally speaking, this mechanism is comprised of negative feedback loops of transcription and translation: activation of a repressor gene results in its later repression by its own protein product, and the instability of this repressor insures this repression is short lived, so that a new cycle can begin.

- In mammals, the principal activators within this system are the CLOCK and BMAL1 proteins and their homologs, which dimerize and bind to certain elements to activate transcription of a large number of circadian genes.

- Among these circadian genes are loci encoding the PERIOD and CRYPTOCHROME families of repressor proteins (PER1-3 and CRY1-2), whose products multimerize and suppress the CLOCK: BMAL1 activating complex. At each of these steps, additional precision and regulatory finesse is achieved through interaction with a wide range of auxiliary proteins: kinases that phosphorylate clock proteins to modify their stability or activity.

Chronopharmacological techniques ensure that drug levels in the blood are within therapeutic ranges during periods of maximal disease severity. An example of this is seen in how evening doses of antihypertensive therapy can be used to prevent morning rises in blood pressure. The evening dose of the drug may thus be well timed with diurnal changes in blood pressure, preventing diurnal worsening of hypertension. In addition, medications may have a different effect based on the timing of the dose. for example, efficacy of ketamine, has been shown to have varying efficacy based on the timing of dose despite reaching equivalent plasma concentrations, giving rise to the theory that some of these diurnal effects may be due to changes in receptors or secondary messenger systems. Chronotherapy may prevent up- or down-regulation of receptors during periods of lesser need allowing optimal efficacy during periods of disease exacerbation. Considering the wide scope of circadian (patho-) physiology, it is logical that the pharmacodynamics and pharmacokinetics (PK/PD) of many drugs would be circadian, and therefore that drug efficacy and safety profiles would also vary with time of day. Nevertheless, this variation is only seldom considered by clinicians, drug developers, or regulators. In part, this apathy may stem from a lack of insight into the molecular mechanisms governing this control. However, two decades of intensive research have uncovered a wealth of information not only about basic mechanisms of circadian clocks, but also about how they interact with physiology and disease. Below, we review this knowledge on cellular and systems levels, and then consider its implications for pharmaceutical intervention.

Circadian control of cellular physiology

Whereas SCN clocks are entrained by light, and peripheral clocks are entrained by indirect and hormonal cues, individual aspects of cellular physiology are in turn directed by both local and central clocks through a variety of mechanisms. One fundamental mechanism is via transcription: in total, about ten percent of all transcripts in each tissue are regulated in circadian fashion. In large part, this regulation occurs through the same *cis*-acting promoter

elements that direct the rhythmic transcription of clock genes themselves, for example the E-boxes that serve as platforms for activation by CLOCK and BMAL1, and the RRE-elements that respond to REV-ERB proteins. Regulation of additional genes can occur through cascades of clock-regulated transcription factors. Among the best-studied are the PAR-bZIP family of factors: DBP, TEF, and HLF. The D-elements to which they bind control circadian expression of several families of genes, including liver metabolic regulators critical to circadian control of pharmacokinetics for a wide variety of drugs. Modeling studies suggest that simple combinations of these three elements – E-boxes, D-boxes, and RRE-elements, each with maximal occupancy at a certain time of day – can direct circadian transcription in any phase, and are responsible for a large portion of circadian transcription directed by cellular clocks.

Nevertheless, this mechanism represents only a portion of circadian transcription in living animals. Experiments in mice lacking functional clocks in specific tissues show that only a portion of circadian gene expression is abolished by such manipulations, while another portion persists because it is systemically driven. A portion of this transcription likely arises through rhythmic activity of the hypothalamic-pituitary-adrenal axis, and another portion through circadian stimulation of action/SRF signaling by unknown ligands. Additional contributions likely arise from heat shock signaling and immune signaling, also regulated by time of day. In all four cases, specific externally-activated transcription factors bind to *cis*-acting elements to drive transcription of certain genes. For example, rhythmic glucocorticoid production results in rhythmic activation of glucocorticoid receptor, which binds to cognate GREs (glucocorticoid receptor elements) to activate or repress transcription. Likewise, circadian body temperature variation results in rhythmic occupancy of heat shock elements (HSEs). The result is circadian transcription of specific genes due to cell-extrinsic influences, and independent of the circadian clockwork present in that cell or tissue.

In addition to circadian transcription recent research has unearthed extensive evidence of circadian posttranscriptional regulation in mammals – including translational control, control of transcription termination and/or elongation, and to a lesser extent circadian control of splicing. Thus, the actual number of transcripts showing circadian abundance is significantly greater than the number of genes transcribed in circadian fashion, and the number of proteins that are expressed in circadian fashion is greater than the number of transcripts where this question has been addressed. Major signaling molecules like cAMP show circadian variations

that both control clock output and play a role within the clock, and recent links between clocks and sirtuins suggest a similar influence of redox potential. Finally, a significant fraction of histone post-translational modifications vary in circadian fashion at a large number of loci. Altogether, through a myriad of different mechanisms, a significant amount of cellular physiology is regulated by the circadian clock.

One case of such regulation meriting special attention is the circadian regulation of the cell cycle and DNA repair. Given the central importance of cell cycle deregulation to cancer – a disease treated separately below – it is easy to understand why circadian control of cell division in adult animals could be of central importance to clinical pharmacology. In fact, multiple studies have documented circadian or diurnal regulation of cell division, both *in vivo* during rodent liver regeneration and *in vitro* in cultured cells. Even in humans, skin blister transcriptional profiling suggests a similar link. Moreover, multiple direct connections have been established between the circadian clock and cell cycle checkpoints, including via the checkpoint proteins WEE1, p21-WAF, and CHK1/2, and by control of circadian transcription of the *p16-Ink4a* locus through the clock-associated NONO protein. In the latter case, this circadian interaction has been demonstrated to be directly important for tissue regeneration, Related to circadian cell cycle control, extensive regulation of DNA damage repair by the circadian clock has also been documented, and this control would directly influence susceptibility to cancer.

Neurotransmitters and Circadian Behavior

Nearly all behaviors show diurnal patterns of activity. In most cases, these oscillations manifest themselves independently of the external environment or the sleep-wake cycle. For example, longterm memory shows a direct dependence on the circadian oscillator: Rodents and humans learn better at certain times of day than others, and mice with functional circadian systems learn better than those without Similarly, anxiety behaviors show a clear diurnal pattern that is modulated both by the sleep-wake cycle and by the circadian oscillator, and these behaviors are elevated in mice lacking the Period clock genes. Even perception of multiple types of pain varies in a circadian fashion in both humans and animal models. The likely basis for these circadian variations is that virtually all major neurotransmitter systems show either marked circadian variations or clock interactions. For example, circadian variations in opioid receptor abundance, as well as in the abundance of natural opioids themselves, have been reported numerous times over the past two decades. The serotonergic

system shows clear ultradian variations corresponding to sleep state, but these faster oscillations interact markedly with the circadian clock, and serotonergic signals appear necessary for the integration of circadian information by the basal forebrain in controlling sleep timing. In the cholinergic system, numerous circadian variations have also been documented. For example, after a sustained attention task with daily repetition, a daily increase in prefrontal cholinergic neurotransmission is observed even in the absence of the task. In general, the cholinergic system plays a critical role in this type of circadian "time-stamping" behavior. This behavior is sustained by the circadian release of acetylcholine during the active phase of many mammals, accompanied by an increase in choline acetyltransferase and a decrease in acetylcholinesterase activity. Globally, the expression of muscarinic acetylcholine receptors shows a pattern inverse to that of acetylcholine availability, with increased abundance during the quiescent phase of the 24-h day, irrespective of activity per se. Examination of the dopaminergic system also shows a diurnal pattern of dopamine abundance within the rodent forebrain. Interestingly, this circadian expression appears necessary for the oscillation of the circadian clock gene Per2 in forebrain neurons, suggesting that dopamine plays a role in mediating circadian information to this brain region. Multiple other neurotransmitters show circadian abundance that strongly interacts with the sleep-wake cycle. For example, adenosine shows circadian variations within the brain that are believed to be sleep-wake dependent. More broadly, purinergic signaling shows a strong circadian component and interacts directly with the circadian machinery through ATP, cAMP, and AMP. The hypocretin/orexin system also has circadian variation that regulates REM sleep in particular. Circadian release of γ-aminobutyric acid (GABA) and glutamate—the principal inhibitory and excitatory neurotransmitters of the brain, respectively—controls not only behavior but also hypothalamic hormone release, which regulates many aspects of physiology.

Circadian Immune Regulation

A second prominent pharmacological target with strong circadian regulation is the immune system. Diurnal variations in white blood cell count and susceptibility to endotoxic shock have long been documented. However, recent research shows that cell-autonomous clocks within immune cells themselves direct variation in a large number of circadian immune parameters. For example, the response of T cells to stimulation varies in a circadian fashion, and macrophages, in turn, stimulate immune responses in an equally diurnal fashion with their own clocks. By contrast, far fewer reports of circadian B cell activity exist, and, indeed,

the oscillations documented in circadian gene expression in peripheral blood mononuclear cells are mostly much lower in amplitude than those observed in other tissues such as the liver. The consequences of pervasive circadian regulation of immune function are numerous and range far beyond the aforementioned diurnal variation in infective susceptibility. For example, a pronounced circadian oscillation of blood clotting has long been known and is supported by circadian variation in factors ranging from platelet aggregation and adhesion to the actual expression of clotting factors such as PAI-1 (plasminogen activator inhibitor-1). Circadian clocks also regulate circulation of many immune cells such as hematopoietic stem cells. Finally, circadian immune regulation results in diurnal variations in related immune parameters such as inflammation, which plays a strong role in circadian variation in many diseases.

CHRONOBIOLOGICAL IMPLICATIONS FOR DRUG TREATMENT

To what extent has the knowledge presented above translated to effective pharmaceutical interventions? The most obvious examples of successful chronotherapy are ones with obvious time-ofday-dependent symptoms. Treatment of bronchial asthma has been tuned to result in maximum plasma levels when dyspneas most frequently occur and therefore to alleviate symptoms most effectively. Similarly, blood pressure shows a sharp peak in the early morning, coinciding with the peak for cardiovascular events, and an extended trough during the night. Both healthy normotensive patients and those suffering from essential hypertension exhibit this variation. The L-type calcium channel blocker verapamil, for example, uses an extended-release formulation to result in therapeutically effective plasma levels in the early morning, after bedtime oral administration. In addition, such delayed-release drugs have been beneficial for hypertensive patients who do not show a nocturnal dip in blood pressure, so-called nondippers. Nondipping is a risk factor for congestive heart failure even in clinically normotensive subjects. As mentioned above, not only are PK/PD parameters modulated by time of day, but drug metabolism is as well. For example, over-the-counter acetaminophen [analgesic N-acetyl-paminophenol (APAP)] is a leading cause of drug-induced liver failure in the United States. APAP toxicity is dependent on generation of N-acetyl-p-benzoquinone imine (NAPQI) by the CYP P450 system of the liver, mainly CYP2E1. APAP toxicity is time-of-day dependent, but liver-specific ablation of the clock in mice blunts this rhythm (R. Dallmann, J.P. Debruyne & D.R. Weaver, submitted manuscript).

Implications for Drug Discovery and Development

Classically, the drug discovery process is preceded by the validation of a given target. The mechanism of action is established and molecular targets defined. Taking diurnal changes of relevant parameters into account might mean significantly higher costs because the same experiments might have to be conducted at multiple times of day in order to assess if, for example, a certain type of receptor or protein is expressed only at a specific time of day. However, online resources can be mined for information about the circadian expression of a given transcript or metabolite. A special case involves the quest for drugs against aging-related diseases. As do human beings, rodent species typically used in these assays exhibit attenuated circadian rhythms. Thus, the PK/PD profile and target availability could change during the course of aging. Once the target is confirmed and the lead optimization process has started, the properties of the novel chemical entities are evaluated and selected. Typically, CYP P450 induction and inhibition in human and rodent primary hepatocytes are tested. This testing might introduce bias toward only one phase of the circadian cycle because the cells that are used to evaluate the compounds contain a functional cell-autonomous clock that can influence drug metabolism, as detailed above. The CACO-2 monolayer assay is an industry standard used not only to predict absorption after oral application through the intestinal barrier but also to assess interactions with important transporters such as P-glycoprotein. Interestingly, like the intestinal barrier itself, the human tumor–derived CACO-2 cells have a functioning clock that directly controls expression levels of Mdr1.

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