Pharmacology & Therapeutics 202 (2019) 72–90

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Chronopharmacological strategies focused on chrono-drug discovery

Shigehiro Ohdo *, Satoru Koyanagi, Naoya Matsunaga

Department of Pharmaceutics, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan

ARTICLE INFO

Available online 5 June 2019

Keywords:
Chrono-DDS
Chronopharmacokinetics
Chronopharmacology
Chronotherapy
Clock gene

ABSTRACT

In mammals, the circadian pacemaker resides in the paired suprachiasmatic nuclei (SCN) and influences a multitude of biological processes, including the sleep–wake rhythm. Circadian rhythms regulate diverse physiologic processes, including homeostatic functions of steroid hormones and their receptors. Perturbation of these rhythms is associated with pathogenic conditions such as cancer, metabolic syndrome, cardiovascular disease, sleep disorder and depression. Clock genes ultimately control a vast array of circadian rhythms involved in physiology and behavior. They regulate several diseases described above. Chronotherapy is especially relevant when the risk and/or intensity of symptoms of a disease vary predictably over time. The effectiveness and toxicity of several drugs vary depending on the dosing time. Such chronopharmacological phenomena are influenced by not only the pharmacodynamics but also the pharmacokinetics of a medication. The underlying mechanisms are associated with the 24-h rhythms of biochemical, physiological, and behavioral processes under the control of the circadian clock. Identifying a rhythmic marker based on the molecular clock for choosing dosing time can lead to the progress and diffusion of chronopharmacotherapy. To monitor the rhythmic markers such as clock genes, it might be useful to choose the most appropriate time of a day for the administration of a drug, to increase its therapeutic effects and/or reduce its side effects. On the contrary, several drugs affect the molecular clock and alter the 24-h rhythms of various processes. Alterations in rhythmicity are sometimes associated with therapeutic effects, or it might lead to illness and altered homeostatic regulation. Furthermore, to produce new rhythmicity by manipulating the molecular clock of organs by rhythmic administration of drugs at altered feeding schedules appears to lead to a new concept of chronopharmacotherapy. An approach to increase the efficiency of pharmacotherapy is administering drugs at times when they are best tolerated. From the perspective of pharmaceutics, the application of biological rhythm to pharmacotherapy can be accomplished by the appropriate timing of administration of conventionally formulated tablets and capsules, and also by the use of special drug-delivery system to synchronize drug concentrations to the rhythms in disease activity. New drugs targeting the molecular clock are being developed to manage diseases in human. For instance, novel molecular mechanisms that mediate renal dysfunction in mice with chronic kidney disease (CKD) have been identified by examining the relationship between the circadian clock and CKD aggravation. The inhibition of cell cycle regulatory factor ameliorated renal inflammation in a mouse model of CKD. A novel inhibitor of cell cycle regulatory factor has been identified, supporting the potential utility of cell cycle regulatory factor inhibition in the treatment of CKD. Although malignant phenotypes of triple-negative breast cancer are subject to circadian alterations, the role of cancer stem cells (CSCs) in defining this circadian change remains unclear. The effectiveness of anticancer drugs varies with the circadian dynamics of CSCs, which are regulated by the tumor microenvironmental factors. The finding reveals that the circadian dynamics of CSCs are regulated by the tumor microenvironment and provides a proof of

Abbreviations: 5-FU, 5-fluorouracil; APRPG, Ala-Pro-Arg-Pro-Gly; APRPG-LipADM, adriamycin-encapsulated liposomes modified with the Ala-Pro-Arg-Pro-Gly peptide; ASWD, advanced sleep phase syndrome; bHLH, basic helix-loop-helix; BT, body time; CircAct, rest-activity circadian rhythm; CJL, chronic jet-lag; CKD, chronic kidney disease; CKIε, casein kinase I epsilon; CLIF, cycle-like factor; Cmax, concentration peak height; CRSD, Circadian rhythm sleep disorders; Cyp, cytochrome P-450; DBP, albumin D-site binding protein; DDS, drug-delivery system; DEN, diethylnitrosamine; DMARD, disease-modifying anti-rheumatic drug; DR, delayed release; DSPS, delayed sleep phase syndrome; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; GST, glutathione S-transferases; HPA, hypothalamic-pituitary-adrenal; HRQoL, health-related quality of life; IFN, interferon; IL, interleukin; IR, immediate-release; Ka, absorption rate constant; LD, light/darkness; MetAP2, methionine aminopeptidase 2; MP, methylprednisolone; MR, modified-release; OCD, obsessive-compulsive disorder; PBMC, peripheral blood mononuclear cell; PD, pharmacodynamics; PDGF, platelet-derived growth factor; PEF, peak expiratory flow; PK, pharmacokinetics; RA, rheumatoid arthritis; SCN, suprachiasmatic nuclei; SF-36, Short Form-36; SSRI, selective serotonin reuptake inhibitor; ST segment, connects the QRS complex and the T wave in electrocardiography; TEN, total enteral nutrition; TfR1, transferrin receptor 1; Tf-NGPE, transferrin N-glutaryl-dioleoyl-phosphatidylethanolamine; VEGF, vascular endothelial growth factor; VPA, valproic acid; ZT, zeitgeber time.

* Corresponding author.
E-mail address: ohdo@phar.kyushu-u.ac.jp (S. Ohdo).

https://doi.org/10.1016/j.pharmthera.2019.05.018

principle of its implication for chronotherapy against triple-negative breast cancer. Therefore, we present an overview of the dosing-time-dependent alterations in therapeutic outcome and safety of a drug and the regulatory system of biological rhythm from the perspective of clock genes and the possibility of pharmacotherapy based on the molecular clock.

Contents

1. Introduction

According to the conventional method of classifying pharmaceutical variations, there are two major classes of variabilities, namely, interindividual and intraindividual variabilities. Basic pharmacotherapeutic studies have focused only on the interindividual variability of drug pharmacodynamics and pharmacokinetics (PD/PK). The pharmacogenomic/pharmacogenetic studies have elucidated the molecular mechanism of interindividual variability at different levels ranging from different heritable chromosomes to protein polymorphism due to point mutations among species of the same gene at the translation and post-translation stages (Sissung, English, Venzon, Figg, & Deeken, 2010; Zhou et al., 2008). According to this traditional approach, diseases emerge due to either increased or decreased genetic expression of certain molecular targets. This hypothesis did not consider the intraindividual variability. Moreover, it was before the discovery of the clock and clock-controlled genes in mammals in 1997, enabling practitioners to use medications more effectively and safely (Koster, Rodin, Raaijmakers, & Maitland-van der Zee, 2009; Tei et al., 1997). The intraindividual and interindividual variabilities should be considered to further improve rational pharmacotherapy. This is because many drugs vary in their potency and/or toxicity associated with the rhythmicity of biochemical, physiological, and behavioral processes (Duncan Jr., 1996; Halberg, 1969; Smolensky & Labrecque, 1997; Ohdo, 2010; Ohdo, Koyanagi, & Matsunaga, 2010; Ohdo, Koyanagi, Suyama, Higuchi, & Aramaki, 2001; Reinberg & Halberg, 1971; Youan, 2004). Theoretically, it has been argued that drug administration at certain times of the day can improve the outcome of pharmacotherapy.

In all living organisms, one of the most indispensable biological functions is the circadian clock in the suprachiasmatic nuclei (SCN), which acts as a multifunction timer to regulate homeostatic systems such as sleep and activity, hormone levels, appetite, and other functions with a 24-h cycle (Chang & Reppert, 2001; Moore & Eichler, 1972; Sakamoto et al., 1998; Silver, LeSauter, Tresco, & Lehman, 1996; Ueyama et al., 1999). Biological rhythms not only affect the physiological functions, but also the pathophysiology of diseases (Duncan Jr., 1996; Halberg, 1969; Ohdo, 2010; Ohdo et al., 2001; Ohdo et al., 2010; Reinberg & Halberg, 1971; Smolensky & Labrecque, 1997; Youan, 2004). The effectiveness and toxicity of several drugs vary depending on the dosing time associated with the 24-h rhythm of biochemical, physiological, and behavioral processes under the control of the circadian clock. Chronopharmacology is an investigative science that elucidates the biological rhythm dependency of medications. Chronopharmacological phenomena are influenced by not only the PD, but also the PK of a medication. An understanding of the 24-h rhythm in the risk of disease and evidence of 24-h rhythm dependency of drug PK, effects, and safety constitute the rationale for pharmacotherapy. Chronotherapy is especially relevant when the risk and/or intensity of symptoms of a disease vary predictably over time as exemplified by allergic rhinitis, arthritis, asthma, myocardial infarction, congestive heart failure, stroke, and peptic ulcer (Duncan Jr., 1996; Halberg, 1969; Ohdo, 2010; Ohdo et al., 2010; Smolensky & Labrecque, 1997). Pharmaceutical companies have focused on investigating the underlying mechanisms and conducting multicenter clinical investigations involving numerous patients in order to devise chronotherapeutic interventions with a variety of medications. From the perspective of pharmaceutics, the application of biological rhythm to pharmacotherapy might be accomplished by the appropriate timing of conventionally formulated tablets and capsules and by the use of special drug-delivery system (DDS) to synchronize drug concentrations with the rhythms in disease activity (Duncan Jr., 1996; Smolensky & Labrecque, 1997).

The clock genes have been identified to ultimately control a vast array of circadian rhythms involved in physiology and behavior (Balsalobre et al., 2000; Beato, Herrlich, & Schutz, 1995; Jin et al., 1999; Kalsbeek, van Heerikhuize, Wortel, & Buijs, 1996; Kume et al., 1999; Ripperger, Shearman, Reppert, & Schibler, 2000; Shigeyoshi et al., 1997; Zylka, Shearman, Weaver, & Reppert, 1998). The circadian rhythms regulate diverse physiologic processes, including homeostatic functions of steroid hormones and their receptors. Perturbation of these rhythms is associated with pathogenic conditions such as depression, diabetes, and cancer. The clock gene regulates several diseases such as cancer, metabolic syndrome, and sleep. CLOCK mutation affects the expression of not only the rhythmic genes, but also the non-rhythmic genes. The knowledge of intra- and inter-individual variabilities of

molecular clock should be applied in clinical practice (Jones et al., 1999; Maemura et al., 2000; Shimba et al., 2005; Toh et al., 2001). Monitoring of rhythm, overcoming rhythm disruption, and manipulating rhythm from the view point of molecular clock are essential to improve the progress and diffusion of chronopharmacotherapy. Such an approach should be achieved by overcoming the new challenges in DDS that match the circadian rhythm (Chrono-DDS) (Duncan Jr., 1996; Ohdo et al., 2001). Recent studies on pharmacotherapy have focused on gene delivery and antibody delivery targeting specific molecules for some diseases. The clock genes should also be one of the important candidates.

The aim of this review is to (1) provide an overview of the dosing time-dependent alterations in therapeutic outcome and safety of a drug, (2) elucidate the underlying mechanisms and usefulness from the viewpoint of chronopharmacology and chronotherapy, and (3) summarize the regulatory system of biological rhythm with respect to clock genes and the possibility of pharmacotherapy based on the intra- and inter-individual variabilities of clock genes.

2. Biological time and molecular clock

The biological time structure describes the sum of non-random and thus predictable time-dependent biologic changes, including, changes in growth, development, and aging, and a spectrum of rhythm with different frequencies (Halberg, 1969; Reinberg & Halberg, 1971). Basically, the concept of biological time is based on homeostasis, which is maintained in relative constancy over time by specific feedback mechanisms. However, important findings on biological rhythms clearly reveal that biological functions are not static over time. Particularly, biological functions are variable in a predictable manner as rhythms of defined periods. Rhythms of very short period, in the order of seconds or so, are evident in electrocardiographic and encephalographic tracings. Ultradian rhythms of periods in the range of 30 min to 20 h are observed in different endocrine glands and sleep stages. The rhythms with about 24-h period (circadian) have been the most investigated with applications in clinical medicine. Longer period rhythms of seven days (circaseptan), a month (circatrigintan), and a year (circannual) are also known. The monthly rhythms include the menstrual cycle in sexually mature women. Many of these rhythms appear to be genetically fixed and thus endogenous in nature. Endogenous rhythms may or may not be adjusted with respect to their timing by environmental factors, the so-called synchronizers. Chronobiology is a branch of science objectively quantifying and investigating mechanisms of biologic time structure, including rhythmic manifestations of life.

The SCN of the anterior hypothalamus are the site of the circadian pacemaker in mammals (Jin et al., 1999; Moore & Eichler, 1972; Tei et al., 1997) (Fig. 1). Like any timing system, the circadian clock is made up of three components, namely, an input pathway adjusting the time, a central oscillator generating the circadian signal, and an output pathway manifesting itself in circadian physiology and behavior. Considerable research has shown that the inherited period of the human pacemaker clock is not precisely 24 h. In fact, in most people, it is a little longer, close to 25 h. Environmental time cues termed synchronizers or zeitgebers, the strongest one being the daily light–dark cycle occurring in conjunction with the wake–sleep routine, set the inherited pacemaker circadian time-keeping systems to 24 h each day. The daily changes in light intensities are considered the major environmental cue involved in circadian entrainment. Light signals are perceived by photoreceptor cells in the retina and transmitted to neurons of the SCN via the retinohypothalamic tract. The clock genes ultimately control a vast array of circadian rhythms involved physiology and behavior (Jin et al., 1999; Moore & Eichler, 1972; Tei et al., 1997). They are expressed in not only the SCN, but also other brain regions and various peripheral tissues. Such a cascade of clock genes might contribute to the organization of biological rhythms in the whole body. The clock genes regulate molecules associated with diseases, PD, and PK (Ohdo, 2010; Ohdo et al., 2010). However, the mechanisms employed by the circadian output pathways are poorly understood, but they are likely to involve both nervous and humoral signals.

The clock genes control the circadian rhythms involved in physiology and behavior (Jin et al., 1999). Three mammalian clock genes (*Per1*, *Per2*, and *Per3*) are rhythmically expressed in the SCN. *Per1* and *Per2* are induced in response to light (Zylka et al., 1998). In particular, the induction of *Per1* is considered an initial event in light-induced resetting and entrainment of the circadian biological clock (Shigeyoshi et al., 1997). The transcriptional machinery of the core clockwork regulates a clock-controlled rhythm as shown in Fig. 1 (Jin et al., 1999; Moore & Eichler, 1972; Tei et al., 1997). Namely, CLOCK-BMAL1 heterodimers act via an E box enhancer to activate the transcription of *Pers*, *vasopressin*, and *Dbp* mRNA, showing a specific output function (Jin et al., 1999; Kalsbeek et al., 1996; Ripperger et al., 2000). This activation can be inhibited by the PER and CRY proteins (Kume et al., 1999). The circadian rhythm of *Pers* mRNA expression is discovered in not only the SCN

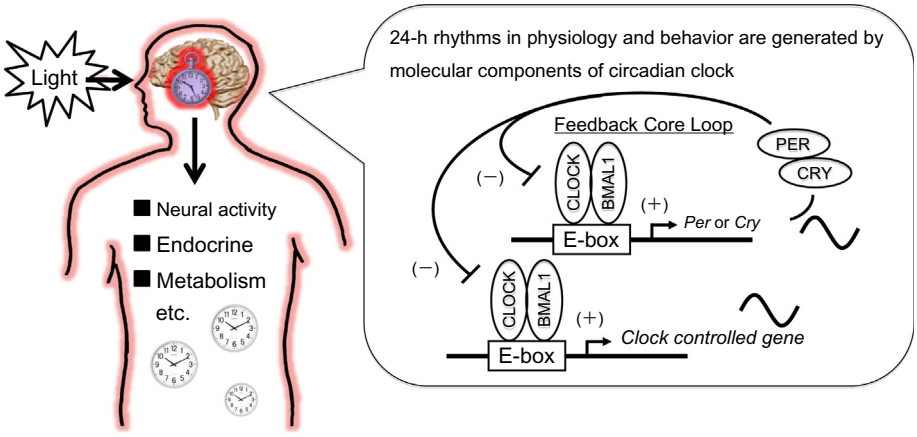

Fig. 1. Schematic diagram of the circadian rhythm in mammals and simplified model of the dual regulation of a core feedback loop (Chang & Reppert, 2001; Koster et al., 2009; Moore & Eichler, 1972). The SCN of the anterior hypothalamus is the site of the circadian pacemaker in mammals. Light-signals, the most important entrainment other than feeding schedule, drugs, and social interaction, are perceived by photoreceptor cells in the retina and transmitted to neurons of the SCN via the retinohypothalamic tract. Clock genes are expressed not only in the SCN, but also in other brain regions and various peripheral tissues. Such a cascade of clock genes may contribute to the organization of biological rhythms in the whole body. The mechanisms employed by circadian output pathways are likely to involve both nervous and hormonal signals. CLOCK and BMAL1 heterodimers activate clock genes and clock-controlled genes transcription. The PER and CRY proteins shut down CLOCK-BMAL1 upregulation in the nucleus, forming a negative feedback loop. The phosphorylation of PER1 (period) and PER2 by CKIε (casein kinase I epsilon) may regulate their cellular location and stability. Clock-controlled genes products including DBP (D-element binding protein) and AVP (arginine vasopressin) transduce the core oscillation to downstream output systems.

but also other tissues (Sakamoto et al., 1998). The circadian rhythm in the peripheral tissue is governed by that in the SCN, as the circadian rhythm of physiological functions and *Pers* mRNA expression was abolished in SCN-lesioned rats (Sakamoto et al., 1998) and *Clock* mutant mice (Jin et al., 1999). Such a cascade of clock genes might contribute to the organization of biological rhythms in the whole body. The plasma glucocorticoid levels show a circadian rhythm via the hypothalamic-pituitary-adrenal (HPA) axis under the control of the SCN. Glucocorticoids regulate various physiological responses and developmental processes by binding to and modulating the transcriptional activity of their cognate nuclear receptor (GR) (Balsalobre et al., 2000; Beato et al., 1995). A transit induction of *Per1* and *Dbp* mRNA levels was observed by a single administration of dexamethasone (Balsalobre et al., 2000). Glucocorticoid hormones are particularly attractive candidates, as they are endogenous substances and play an important role in the entrainment of peripheral oscillators but not SCN (Balsalobre et al., 2000). However, the mechanisms employed by circadian output pathways are poorly understood, but are likely to involve both nervous and humoral signals (Silver et al., 1996; Ueyama et al., 1999). Moreover, the regulatory system of biological rhythm should be clarified in detail from the viewpoint of clock genes.

3. Chronobiology of disease occurrence and disease pathogenesis from viewpoints of molecular clock

Chronotherapeutic approach is based on the presence of 24-h rhythms in physiological functions and diseases. A study has showed the approximate peak time of 24-h rhythms in diurnally active human beings (Smolensky & Labrecque, 1997). The peak in serum cortisol, aldosterone, testosterone, platelet adhesiveness, blood viscosity, and NK-cell activity is observed during the initial hours of daytime. The hematocrit level is the highest and airway caliber (FEV1) is the best around the mid and afternoon hours, respectively. The level of insulin, cholesterol, triglycerides, platelet numbers, and uric acid peaks later during the day and in the evening. The rhythms of basal gastric acid secretion, white blood cells (WBCs), lymphocytes, prolactin, melatonin, eosinophils, adrenal corticotrophic hormone (ACTH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) shows a peak at specific times during the nighttime.

The 24-h rhythms in the processes that affect the pathophysiology of diseases cause prominent day–night patterns in the manifestation and severity of many medical conditions as reported previously (Smolensky & Labrecque, 1997). The onset of migraine headache frequently occurs in the morning around the time of awakening. Sneezing, runny nose, and stuffy nose in allergic and infectious rhinitis are the worst in the early morning. The symptoms of rheumatoid arthritis (RA) are the worst in the early morning, while those of osteoarthritis are the worst later in the day. The ischemic events, chest pain, and ST segment (connects the QRS complex and the T wave in electrocardiography) depression of angina are the strongest during the initial 3–5 h of daytime. The incidence of thrombotic and hemorrhagic stroke is the highest in the morning around the time of commencement of diurnal activity. The morbid and mortal events of myocardial infarction are the highest during the initial hours of daytime. The pain and gastric distress at the onset and acute exacerbation of peptic ulcer disease are most likely in the late evening and early morning. The seizures of epilepsy are common around sleep onset at night and offset in the morning. The symptoms of congestive heart failure are worse at night. The manifestation of ST-segment elevation in Prinzmetal's angina is the most frequent during the middle to later half of the nighttime. The risk of asthma attack is the greatest during night. Chronotherapeutic approach is based on the presence of 24-h rhythms in physiological functions and diseases. The regulatory mechanisms underlying the 24-h rhythm of physiological functions and diseases should be clarified from the viewpoint of clock genes.

Sleep disorder in human is associated with a genetic mutation affecting the circadian clock function. Familial advanced sleep-phase syndrome (FASPS) has been demonstrated for three families (Jones et al., 1999; Toh et al., 2001). Affected individuals experience early evening sleepiness (around 19:30 h) and early morning awakening (around 04:30 h). Individuals with FASPS have a circadian period approximately an hour shorter than the normal one. Taking one of the FASPS families, Toh et al. used multiple sets of dense genomic markers to map the associated mutation and clarified that the mutant gene is *hPer2*, the human homolog of mPer2 (Toh et al., 2001). The *hPer2* mutation substitutes serine 662 with a glycine (S662G). This occurs in the region of hPER2 homologous to the casein kinase I epsilon (CKIε)-binding region of mPER1 and mPER2. Serine 662 is in fact a part of the consensus CKIε phosphorylation site, and the S662G substitution renders the mutant protein less readily phosphorylated by CKIε than the wild-type hPER2 in vitro. Thus, a variation in human sleep behavior can be attributed to a missense mutation in the clock component, hPER2, which alters the circadian period.

BMAL1 is a transcription factor controlling the circadian rhythm and contributes to the regulation of adipose differentiation and lipogenesis in mature adipocytes (Shimba et al., 2005). BMAL1 is also associated with metabolic syndrome. The level of *Bmal1* mRNA increases during adipose differentiation in 3T3-L1 cells. The knock-down of Bmal11 expression in 3 T3-L1 cells allowed the cells to accumulate only minimum amounts of lipid droplets in the cells. Adenovirus-mediated expression of *Bmal1* in 3T3-L1 adipocytes resulted in the induction of several factors involved in lipogenesis. The promoter activity of these genes was stimulated in a BMAL1-dependent manner. These factors show a circadian rhythm in mice adipose tissue. Furthermore, the overexpression of BMAL1 in adipocytes increased lipid synthesis. In white adipose tissues isolated from mice, BMAL1 was more highly expressed in the adipocyte fraction than in the stromal-vascular fraction. Bmal1 knockout mice embryonic fibroblast cells failed to differentiate into adipocytes. Adding BMAL1 via adenovirus gene transfer restored the ability of Bmal1 knockout mice embryonic fibroblast cells to differentiate into adipocytes. Thus, BMAL1 plays important roles in the control of adipogenesis and lipid metabolism activity in mature adipocytes.

The onset of myocardial infarction occurs frequently in the early morning, and it might partly result from the circadian rhythm of fibrinolytic activity. A circadian rhythm is demonstrated for the PAI-1 activity (Maemura et al., 2000). The basic helix-loop-helix (bHLH)/PAS domain transcription factors play a crucial role in controlling the biological clock that regulates the circadian rhythm. A novel bHLH/PAS protein cycle-like factor (CLIF) shares high homology with *Drosophila* CYCLE, one of the essential transcriptional regulators of the circadian rhythm. It is expressed in endothelial cells and neurons of the brain, including the SCN. CLIF has been isolated from human umbilical vein endothelial cells. In endothelial cells, CLIF forms a heterodimer with CLOCK and up-regulates the *Pai-1* gene through the *E*-box sites. Furthermore, PER2 and CRY1 inhibit the activation of *Pai-1* promoter by the CLOCK: CLIF heterodimer. Namely, CLIF regulates the circadian rhythm of the *Pai-1* gene in endothelial cells. The results potentially provide a molecular basis for the morning onset of myocardial infarction.

4. Relevance of chronobiology and chronopharmacologic concepts to chronopharmacodynamics and chronopharmacokinetics

Chronopharmacology deals with the application of chronobiological approaches to pharmacological phenomena (Smolensky & Labrecque, 1997). Chronobiological methodology involves less risk of error and/or false information than that of the conventional homeostatic approach. Chronothrepy is a term to refer to the use of a chronopharmacological approach to clinical treatment. Both chronopharmacology and chronotherapy include chronotoxicity and chronoefficacy. Chronotoxicology describes undesired or harmful effects of chemical, physical, or other agents including poisons, pollutants, and overdoses of drugs upon

alteration of biologic temporal characteristics and as a function of biologic timing. Chronotherapy involves cure or prevention of diseases, with proper regard to temporal characteristics; for example, corticosteroid therapy is timed to simulate the adrenocortical cycle in Addison's disease. The following are some terms associated with chronopharmacology.

Chronopharmacokinetics describes biologic time-related changes in the PK of an agent quantified by parameters of one or several curve patterns. Chronopharmacokinetic studies have been conducted for many drugs in an attempt to explain chronopharmacological phenomena and demonstrate that the time of administration is a possible factor of variation in the PK of a drug. Time-dependent changes in drug PK might proceed from the 24-h rhythms at each process, e.g., absorption, distribution, metabolism, and elimination. Thus, the 24-h rhythms in gastric acid secretion and pH, motility, gastric emptying time, gastrointestinal blood flow, drug protein binding, liver enzyme activity and/or hepatic blood flow, glomerular filtration, renal blood flow, urinary pH, and tubular resorption might play a role in such pharmacokinetic variations (Smolensky & Labrecque, 1997). Chronopharmacokinetics can, but may not always, be responsible for the daily variation in drug effects and/or adverse effects.

The therapeutic-to-toxicity ratio of a medication varies predictably according to chronobiological determinants, as exemplified by antitumor medications. The PK and PD of a medication vary depending on the biological rhythms. The goal of pharmacotherapy is hormonal substitution to mimic the rhythmic variation of hormone levels in healthy individuals. Further, on the horizon are drugs to fix altered biological clocks, perhaps a crucial factor in all illness, in the opinion of some physicians. Several examples for chronopharmacotherapy are shown in Table 1 (Alten, 2012; Ohdo, 2010; Smolensky & Labrecque, 1997).

The chronopharmacological concepts in terms drug efficacy and safety have been demonstrated using drug testing and post-approval drug surveillance. The involvement of human circadian rhythms in chronopharmacology has been identified by conventional endpoints of response as shown in classical manifestation during asthma and cancer therapies. The term chronopharmacology refers specifically to predictable-in-time variation in patient sensitivity to the effects of medications due to biological rhythm determinants.

Table 1
Chronotherapy to be incorporated into clinical practice (modification from Smolensky & Labrecque, 1997; Smolensky & Alozo, 1997; Ohdo, 2010; Alten, 2012).

- The morning daily or alternate-day dosing strategy for methylprednisolone that was introduced during the 1960s constitutes the first chronotherapy to be incorporated into clinical practice. (Smolensky & Labrecque, 1997)
- MR (modified-release) prednisone is currently approved in the USA and several European countries, including Italy, for the treatment of adults with moderate--to-severe, active rheumatoid arthritis (RA), particularly when accompanied by morning stiffness. (Alten, 2012)
- Evening, once-daily dosing of specially formulated theophylline tablets for treatment of nocturnal asthma. (Smolensky & Alozo, 1997)
- Before-bedtime administration of verapamil HCL as a unique controlled onset extended-release 24 h dosage form to optimize the treatment of patients with ischemic heart disease and/or essential hypertention. (Smolensky & Labrecque, 1997)
- Evening administration of HMG-CoA-reductase antagonists for the management of hyperlipidemia. Evening, once-daily dosing of conventional H2-receptor antagonist or morning once-daily administration of protonpump antagonist tablet medications for the management of peptic ulcer disease. (Smolensky & Labrecque, 1997)
- Before-bedtime administration of hypnotics for sleep induction and maintenance. (Smolensky & Labrecque, 1997)
- Morning application of testosterone drug-delivery patch systems to achieve a physiologic androgen-replacement therapy. (Smolensky & Labrecque, 1997)
- Programmed-in-time infusion of antitumor medications according to biological rhythms to moderate toxicity and enhance dose-intensity in cancer treatment. (Ohdo, 2010)
- Programmed-in-time administration of tocolytic medication relative to the circadian rhythm in uterine contractility to avert preterm labor and birth. (Smolensky & Labrecque, 1997)

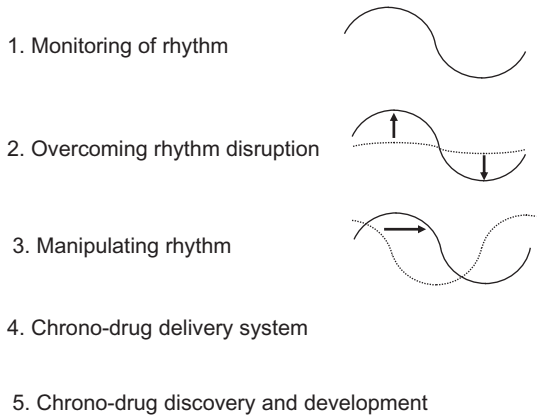

Fig. 2. Schematic diagram of chronotherapeutic strategies based on the monitoring of rhythm, overcoming rhythm disruption, manipulation of rhythm, chrono-drug delivery system and chrono-drug discovery from the viewpoints of molecular clock (Ohdo, 2010). To monitor the rhythmic markers such as clock genes, it might be useful to choose the most appropriate time of a day for the administration of a drug, to increase its therapeutic effects and/or reduce its side effects. On the contrary, several drugs affect the molecular clock and alter the 24-h rhythms of various processes. Alterations in rhythmicity are sometimes associated with therapeutic effects, or it might lead to illness and altered homeostatic regulation. Furthermore, to produce new rhythmicity by manipulating the molecular clock of organs by rhythmic administration of drugs at altered feeding schedules appears to lead to a new concept of chronopharmacotherapy. The chrono-DDS and chrono-drug discovery can help develop new therapeutic strategies for several diseases and provide insights into chronotherapy as a way to optimize current therapies.

A schematic diagram of chronotherapeutic strategies to monitor rhythm, overcome of rhythm disruption, manipulate rhythm, develop chrono-drug delivery system and discover chrono-drug from the viewpoint of molecular clock is shown in Fig. 2 (Ohdo, 2010). The effectiveness and toxicity of several drugs vary depending on the dosing time associated with the 24-h rhythms of biochemical, physiological, and behavioral processes under the control of circadian clock. The drugs for several diseases are administered without regard to the time of the day. The identification of a rhythmic marker for selecting dosing time will lead to further progress and diffusion of chronopharmacotherapy. To monitor the rhythmic markers such as clock genes, it might be useful to choose the most appropriate time of the day for the administration of drugs that might increase their therapeutic effects and/or reduce their side effects. Several drugs cause alterations in the 24-h rhythms of biochemical, physiological, and behavioral processes. The alteration of rhythmicity is sometimes associated with therapeutic effects, or sometimes it may lead to illness and altered homeostatic regulation. Attention should be paid to the alteration of biological rhythm and consider it an adverse effect when it leads to altered regulation of the circadian system, which is a serious problem affecting the basic functioning of living organisms. Furthermore, to produce new rhythmicity by manipulating the conditions of organs by rhythmic administration of several drugs altered feeding appears to lead to a new concept of chronopharmacotherapy (Lewy, 1999; Ohdo, Ogawa, Nakano, & Higuchi, 1996). The chrono-DDS and chrono-drug discovery can help develop new therapeutic strategies for several diseases and provide insights into chronotherapy as a way to optimize current therapies.

The circadian rhythm of serum cortisol in day-active persons shows a peak in the morning around the time of awakening, generally around 08:00 h. Time-dependent differences in the effects of corticosteroid medications have been investigated on adrenal suppression after a single infusion of methylprednisolone (MP) at different times in diurnally active healthy subjects (Ceresa, Angeli, Boccuzzi, & Molino, 1969; Smolensky & Labrecque, 1997). When MP was infused between 00:00 and 04:00 h, cortisol secretion was markedly suppressed. In contrast, when MP was infused during the late afternoon and early evening, between l6:00 and 20:00 h, cortisol suppression was moderate. When

MP was infused between 08:00 and l6:00 h, cortisol secretion remained normal. The dosing time-dependent inhibitory effect of MP on cortisol secretion is due to the circadian rhythm in the inhibition of drug-induced HPA axis function (Reinberg, 1989; Reinberg, Smolensky, D'Alonzo, & McGovern, 1988).

Once-daily dosing of armophylline (600–900 mg/24 h), a sustained-release theophylline, was administered to eight patients suffering from nocturnal asthma either at 08:00 or 20:00 h for eight days in a double-blind, cross-over randomized study (Reinberg et al., 1987). The study variables monitored daily were self-measured peak expiratory flow (PEF), heart rate, oral temperature, self-rated fatigue checked every 2 h during the waking period as well as during spontaneous nocturnal wakening, and duration and subjective characteristics of sleep rated every morning. In addition, the serum theophylline concentration and the variables described above were recorded every 2 h for 24 h on day 8 of each timed treatment span. Dosing at 20:00 h moderated the nocturnal fall; it was only approximately 10% and within the physiologic limits of non-asthmatic persons. Dosing at 08:00 h was associated with a nocturnal dip in the PEF of approximately 20% from the level achieved during diurnal crest. The theophylline concentration peak height (Cmax) was higher and time-to-peak was shorter with dosing at 08:00 h than those at 20:00 h. Dosing at 20:00 h showed a serum theophylline concentration plateau for approximately 12 h. A statistically significant correlation between the PEF and the corresponding in-time serum theophylline concentration was observed with the dosing at 20:00 h, but not with the dosing at 08:00 h. A sustained-release theophylline dosing at 20:00 h controlled the nocturnal dip of bronchial potency with no major side-effects in patients with nocturnal allergic asthma.

Chronotherapy is especially relevant for antitumor agents (Hrushesky, 1985; Levi, 2001; Levi, Zidani, & Misset, 1997). In a randomized multicentre trial involving patients with previously untreated metastases from colorectal cancer, 93 were administered constant-rate infusion and 93 patients were administered chronotherapy via multichannel programmable ambulatory pumps (Levi et al., 1997). The chronomodulated infusion of 5-fluorouracil (5-FU) (peak at 04:00 h), folinic acid (peak at 04:00 h) and oxaliplatin (peak at 16:00 h) was compared with a constant-rate infusion method. The objective response rate was 51% for chronotherapy and 29% for constant-rate delivery. According to this multicentre randomized trial, the most active chronomodulated schedule was also the least toxic. The median survival was 16 months with both modalities, possibly because 24% of the patients crossed over from the flat schedule to chronotherapy. Cumulative peripheral sensory neuropathy with functional impairment was reported in 31% patients on constant delivery and in 16% patients on chronotherapy. Severe stomatitis was observed in 76% of the patients receiving fixed-rate infusion and 14% of those on chronotherapy. The merit of chronomodulated infusion described above was supported by the following evidence. Cisplatin is better tolerated between 16:00 and 20:00 h than when administered at 12-h interval (Levi, 2001). The chronopharmacokinetic finding of cisplatin revealed decreased renal toxicity with evening administration. In human bone marrow, skin, and oral and rectal mucosae, DNA synthesis, a stage of cell division associated with increased susceptibility to S-phase-specific agents, decreased by 50% or more between 00:00 and 04:00 h compared with that during daytime (Levi, 2001). The activity of dihydropyrimidine dehydrogenase in human mononuclear cells increased by 40% around midnight (Levi, 2001). This enzyme brings about the intracellular catabolism of 5-FU and contributes to improved tolerability of 5-FU between 00:00 and 04:00 h. These findings show that the circadian stage at which anticancer drugs are administered to patients should be carefully considered. Two clinical trials compared the toxicity of two dosing times of anthracyclines and cisplatin in 30 patients with advanced ovarian cancer. Both studies demonstrated that doxorubicin is better tolerated at around 06:00 h and cisplatin between 16:00 and 20:00 h than when administered at 12-h intervals (Hrushesky, 1985).

Circadian rhythm sleep disorders (CRSD) are a group of sleep disorders characterized by age-synchronization between a person's biological clock and the environmental 24-h schedule (Ayalon, Hermesh, & Dagan, 2002; Dagan, 2002; Dagan, Yovel, Hallis, Eisenstein, & Raichik, 1998; Wirz-Justice et al., 2000; Wirz-Justice, Cajochen, & Nussbaum, 1997). There are four main types of CRSD, namely, delayed sleep phase syndrome (DSPS) (the most common), advanced sleep phase syndrome (ASWD), non-24-h sleep–wake syndrome (free-running pattern), and irregular (or disorganized) sleep–wake pattern. These disorders lead to harmful psychological and functional difficulties, and certain personality disorders may also be related to them. Typical DSPS was observed during treatment with the selective serotonin reuptake inhibitor (SSRI) fluvoxamine, prescribed for its obsessive-compulsive disorder (OCD) (Hermesh, Lemberg, Abadi, & Dagan, 2001). The delay in falling asleep ranges between 2.5 and 4 h, later than a patient's normal sleep routine. SSRIs have diverse effects on sleep continuity and nocturnal arousals (Duncan Jr., 1996). The SSRI fluoxetine increases arousals and the amount of Stage 1 sleep, and tends to reduce slow wave sleep in patients with depression. Fluvoxamine diminishes sleep continuity more than that by the tricyclic antidepressant desipramine; this may be related to its rapid eye movement sleep suppressing properties. Attention should be paid to the alteration of sleep–wake cycle. It has been found that the psychotropic drug haloperidol can cause CRSD, and this is also true for some cases of minor head trauma. For example, a patient with Gilles de la Tourette syndrome treated with haloperidol, ingested once daily after awakening from sleep, exhibited an irregular sleep–wake pattern with a free-running component of approximately 48 h (Wirz-Justice et al., 2000). Switching to risperidone, ingested once daily after awakening from sleep, was beneficial, resulting in a sleep–wake cycle more synchronized at an appropriate phase to external zeitgebers and fewer nocturnal disturbances. The circadian sleep–wake schedule was fully synchronized when the patient was subsequently treated with melatonin at 2 l:00 h, before intended nocturnal sleep, in addition to risperidone in the morning.

Sleep disorders are a common symptom and characteristic of numerous psycho-pathologies such as depression, anxiety, and post-traumatic stress disorder (PTSD). It should be emphasized that no existing psychopathology is characterized by a sleep disorder of the circadian type. Similar findings reported in patients suffering from other disorders support the hypothesis that the described disruption of the sleep–wake schedule is due to medication rather than illness related. Therefore, it is important to realize that the circadian rhythm-related sleep disorders may be a side effect of psychotropic drugs.

5. Chronopharmacodynamics and molecular clock

Chronergy describes time-dependent effects of drugs on the organism as a whole. It pertains to rhythmic changes in both the desired and undesired effects of medications. It depends on both the chronesthesy of affected biological targets and the chronopharmacokinetics of a given drug.

Chronesthesy describes rhythmic (predictable-in-time) differences in the susceptibility or sensitivity of a biological target (such as, receptors, membrane permeability, cells, tissues, organs, and organ systems) to an agent. It emphasizes predictable, rather than randomly distributed, biologic time-related differences of such targets. When healthy organisms are concerned and metabolic processes are documented, the term chronopharmacodynamics is used by certain authors instead of chronesthesy. Biological rhythms not only affect the pathophysiology of diseases, but also the PK and PD of medications. Biological rhythms at the cellular and subcellular levels can lead to significant dosing-time differences in the PD of medications that are unrelated to their PK (Matsuo et al., 2003; Nakagawa et al., 2006; Ohdo et al., 1997; Ohdo et al., 2000). This phenomenon is termed chronesthesy. Rhythms in receptor number or conformation, secondary messengers, metabolic

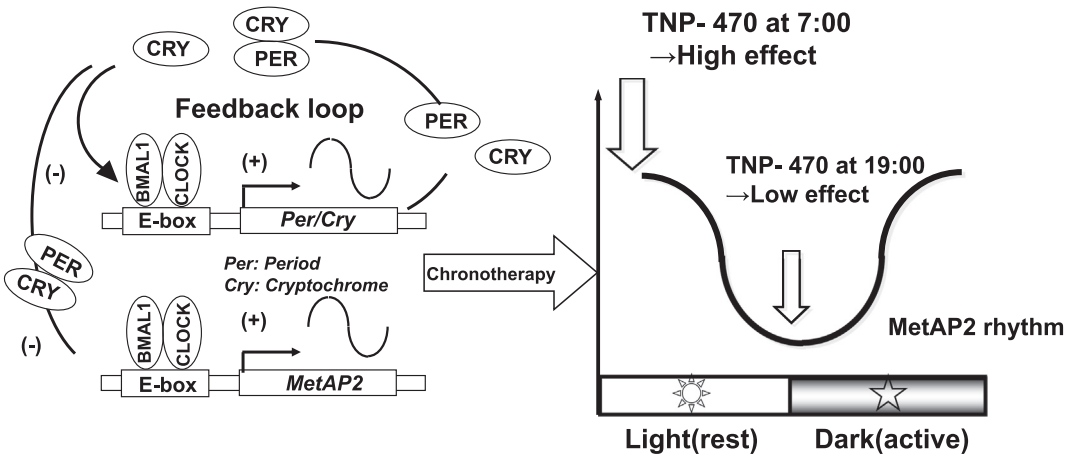

Fig. 3. Simplified model for the molecular clock mechanisms of methionine aminopeptidase 2 (MetAP2) and the dosing time-dependent antitumor efficacy of antiangiogenic agents in mice (Nakagawa et al., 2004). MetAP2 plays an important role in the growth of endothelial cells during the tumor angiogenesis stage. MetAP2 showed a circadian rhythm in implanted tumor masses. The mechanism underlying the circadian rhythm of MetAP2 activity has been investigated in tumor-bearing mice. The 5′ flanking region of MetAP2 includes eight *E*-boxes. The transcription of the MetAP2 promoter is enhanced by the CLOCK:BMAL1 heterodimer, and its activation is inhibited by PER2 or CRY1. Particularly, the circadian rhythm of MetAP2 activity is regulated by the transcription of clock genes within the clock feedback loops. Furthermore, the antitumor efficacy of MetAP2 inhibitor is enhanced by administering the drugs at the time when the MetAP2 activity increases.

pathways, and/or free-to-bound fraction of medications help explain this phenomenon.

Angiogenesis is essential for tumor growth and metastasis. The inhibition of angiogenesis has emerged as a new therapy for cancers. Methionine aminopeptidase 2 (MetAP2) plays an important role in the growth of endothelial cells during the tumor angiogenesis stage. MetAPs showed a circadian rhythm in implanted tumor masses as schematically described in Fig. 3 (Nakagawa et al., 2004). The mechanism underlying the circadian rhythm of MetAP2 activity has been investigated in tumor-bearing mice. The 5′ flanking region of *MetAP2* includes eight *E*-boxes. The transcription of the *MetAP2* promoter is enhanced by the CLOCK:BMAL1 heterodimer, and its activation is inhibited by PER2 or CRY1. Particularly, the circadian rhythm of MetAP2 activity is regulated by the transcription of clock genes within the clock feedback loops. Furthermore, the antitumor efficacy of MetAP2 inhibitor is enhanced by administering the drugs at the time when the MetAP2 activity increases. Hypoxia-induced expression of vascular endothelial growth factor (VEGF) plays a key role in tumor-induced angiogenesis. The level of VEGF mRNA in tumor cells implanted in mice increased substantially in response to hypoxia, but its level showed a circadian rhythm (Koyanagi et al., 2003). Luciferase reporter gene analysis revealed that PER2 and CRY1, whose expression in implanted tumor cells showed a circadian rhythm, inhibit hypoxia-induced VEGF promoter activity. Specifically, the negative limbs of the circadian clock feedback loop periodically inhibit hypoxic induction by VEGF transcription, resulting in the circadian rhythm fluctuation in the mRNA expression of VEGF. Furthermore, the antitumor efficacy of antiangiogenic agents was enhanced by administering drugs at the time when VEGF production increases.

The sensitivity of cancer cells to chemotherapeutic agents varies according to circadian time (Horiguchi et al., 2013). Most chemotherapeutic agents ultimately cause cell death through cell-intrinsic pathways as an indirect consequence of DNA damage. The p53 tumor suppressor gene (TRP53) configures the cell deaths induced by chemotherapeutic agents, but the amount of p53 protein in tumor cells exhibits circadian oscillation. Activating transcription factor-4 (ATF4), a member of the ATF/cAMP response element (CRE)-binding protein family, regulates the circadian accumulation of p53. In murine fibroblast tumor cells, ATF4 induces the circadian expression of p19ARF (Cdkn2a). Oscillation of p19ARF interacts in a time-dependent manner with MDM2, a specific ubiquitin ligase of p53, resulting in a rhythmic prevention of its degradation by MDM2. Consequently, the half-life of p53 protein varies in a circadian time-dependent manner without variation in mRNA levels. The p53 protein accumulates during those times when the p19ARF-MDM2 interaction is facilitated. Notably, the ability of the p53 degradation inhibitor nutlin-3 to kill murine fibroblast tumor cells is enhanced when the drug is administered at those times of day during which p53 has accumulated. This ATF4-mediated circadian accumulation of p53 controls the time-dependent change in the sensitivity of cancer cells to chemotherapeutic agents.

The mammalian target of rapamycin (mTOR) signaling pathway integrates both intracellular and extracellular signals and serves as a regulator of cell growth and survival. The levels of mTOR protein show circadian oscillation in renal adenocarcinoma cells (RenCa) implanted in mice (Okazaki et al., 2014). Consequently, the amount of phosphorylated (active form) mTOR protein is also changed in a circadian time-dependent manner. Fbxw7 acts as an E3 ubiquitin ligase that targets mTOR. The expression of Fbxw7 is under the control of molecular circadian clock. The levels for Fbxw7 mRNA and its protein in RenCa tumor cells exhibit circadian oscillation. The time-dependent changes in the Fbxw7 levels affect the stability of mTOR protein through its degradation process. Notably, administration of the mTOR inhibitor everolimus during the times of day when accumulation of mTOR improves survival of tumor-bearing mice.

Neuropathic pain is chronic condition that often occurs after peripheral nerve injury. One troublesome hallmark symptom of neuropathic pain is hypersensitivity to normally innocuous stimuli. Circadian variations in pain hypersensitivity are common in chronic pain disorders. The dosing time-dependent difference in the anti-allodynic effects of gabapentin is attributable to the circadian oscillation in the DRG and the optimizing its dosing schedule helps to achieve rational pharmacotherapy for neuropathic pain (Kusunose et al., 2010). Neuropathic pain hypersensitivity in sciatic nerve-injured mice shows pronounced circadian alterations, which critically depend on circadian secretion of glucocorticoids from the adrenal glands (Koyanagi et al., 2016). Circadian exacerbation of pain hypersensitivity is mediated by glucocorticoid-induced enhancement of the extracellular release of ATP in the spinal cord, which stimulates purinergic receptors on microglia in the dorsal horn. Serum- and glucocorticoid-inducible kinase-1 (SGK-1) as the key molecule responsible for the glucocorticoid-enhanced release of ATP from astrocytes. SGK-1 protein levels in spinal astrocytes are increased in response to glucocorticoid stimuli and enhance ATP release by opening the pannexin-1 hemichannels. Since adrenal secretion of glucocorticoids is regulated by products of clock gene, circadian

machinery also affects pain hypersensitivity caused by peripheral nerve injury.

Dopamine D3 receptor (DRD3) in the ventral striatum is relevant to motivation and motor functions. The expression of DRD3 in the ventral striatum of mice shows circadian oscillation, which is regulated by molecular components of the circadian clock (Ikeda et al., 2013). The transcription of DRD3 is enhanced by the retinoic acid-related orphan receptor α (RORα), and its activation is suppressed by the orphan receptor REV-ERBα. Brief exposure of cultured cells to high concentration (50%) serum or glucocorticoid induces the rhythmic expression of clock genes. Therefore, serum- or glucocorticoid-treated cells are often employed as an in-vitro model to study the molecular mechanism of circadian clock. The expression of DRD3 oscillated in serum- or dexamethasone-treated cultured cells. The oscillation is abrogated by the downregulation or overexpression of REV-ERBα. DRD3 protein expression in the ventral striatum of mice varies with higher levels during the dark phase. 7-hydroxy-N,N-dipropyl-2-aminotetralin (7-OH-DPAT) is a agonist of DRD3. 7-OH-DPAT -induced locomotor hypoactivity is enhanced when DRD3 proteins is abundant. Molecular interaction between the circadian clock and the function of DRD3 in the ventral striatum modulate the pharmacological actions of compounds targeting DRD3.

Serotonin (5-HT) transporter (5-HTT) plays a key role in the control of 5-HT neuronal activity by reuptaking extracellular 5-HT from the synapse cleft. The anti-immobility effect of fluvoxamine in the forced swimming test increases depending on dosing time. The time-dependent change of SERT mRNA expression and uptake activity in the midbrain is suggested to be the mechanism underlying the 24-h rhythm of anti-immobility effect of fluvoxamine. The mRNA levels of 5-HTT and its uptake activity in the mouse midbrain increases during the dark phase (Ushijima et al., 2012). The expression of ATF4 is under the control of molecular circadian clock and this transcriptional factor is responsible for sustaining circadian oscillations of CRE-mediated gene expression. ATF4 activates *5-HTT* transcription and time-dependently binds to the CRE site in the *5-HTT* promoter in the mouse midbrain. In addition, mutation of the Clock gene disrupts temporal binding of ATF4 to the CRE site in the *5-HTT* promoter. ATF4 connects circadian clock machinery with neuronal 5-HTT function.

6. Chronopharmacokinetics and molecular clock

Time-dependent changes in drug PK might proceed from the 24-h rhythms at each process, e.g., absorption, distribution, metabolism, and elimination. Several physiological factors such as gastrointestinal, cardiovascular, hepatic, and renal changes vary according to the time of a day (Ohdo, 2010; Ohdo et al., 2010). Each process is also influenced by life style such as active-rest cycle, posture and feeding schedule, and physicochemical properties of a drug (lipophilicity or hydrophilicity).

The clock genes are expressed not only in the SCN, but also in other brain regions and various peripheral tissues. As the liver is a major organ of metabolism and detoxification, the knowledge of circadian effects on transcriptional activities that govern the daily biochemical and physiological processes in the liver might play a key role in toxicology. The liver is a biological clock capable of generating its own circadian rhythms (Turek & Allada, 2002). Analysis of relative levels of gene expression in the liver of rats was investigated depending on the of time of the day (Desai et al., 2004). The expression level of 3906 genes was determined using high-density oligonucleotide microarrays. Among them, 30% was highly expressed, whereas 70% was not expressed or the expression was too low to distinguish from the background levels. The maximum estimated changes observed for most rhythmic genes (90%) were <1.5-fold. 67 genes expression was significantly altered depending on the time of the day. These genes are related to DNA binding and regulation of transcription, drug metabolism, ion transport, signal transduction, and immune response.

The circadian rhythm has been demonstrated for six genes involved in the regulation of gene expression by forming transcriptionally active complexes on DNA such as nuclear receptors, retinoic acid receptor-alpha and retinoid X receptors (Desai et al., 2004). Aryl hydrocarbon receptor nuclear translocator works as a transcription factor in diverse signaling events including the response to xenobiotics. The *Pitx2* and *Pitx3* genes also show a circadian expression. These genes encode paired-like homeodomain transcription factors 2 and 3, members of the homeobox gene family, involved in the regulation of other genes and gene products. A significant circadian rhythm has been demonstrated for the expression of cytochrome P-450 4a3 (*Cyp4a3*) and putative *N*-acetyltransferase camello 4 of phases I and II of drug metabolism (Desai et al., 2004). The liver Cyp4a isoforms play a major role in the regulation of renal function by catalyzing the formation of 20-hydroxyeicosatetraenoic acid, which has a potent effect on the renal vasculature and tubular ion transport. This might partly explain the circadian rhythm of renal function and blood pressure. In mouse liver, the *Cyp* isoforms such as *Cyp17*, *Cyp2a4*, *Cyp2e1*, and *Cyp2c22* show a circadian regulation of transcripts. N-Acetyltransferase camello 4 encodes a protein catalyzing the acetylation of aromatic amines and hydrazines. The rhythmic pattern corresponds to the pattern of *Cml2* in mouse liver. The members of phase II drug-metabolizing enzymes such as glutathione S-transferases (GST) and carboxylesterase shows a circadian rhythm. Coordinated rhythmic oscillations in phases I and II components of drug metabolism during the day might account for differential responses to drugs in toxicology. The liver is the major organ of metabolism and endures a flux of metabolites across membranes. A rhythmic gene expression has also been demonstrated for genes involved in solute carrier transporters such as solute carrier family 34 (an insulin-regulated facilitative glucose transporter), and solute carrier family 2 (a phosphate ion transporter) (Desai et al., 2004). A significant circadian rhythm has been demonstrated for those encoding solute carriers such as *Slc12a2*, *Slc16a1*, *Slc19a1*, and *Slc25a11*. Circadian rhythm has been demonstrated for the expression of *Slc10a1*, *Slc22a1*, *Slc27a1*, *Slc2a2*, and *Slc7a2* shows a circadian rhythm in addition to the anion and solute transporters *Abcc2* and *Aqp9*. Furthermore, a significant circadian expression has been demonstrated for genes *Hcn4*, *Trpc4*, *Scn2b*, *Scn4a*, *Chrnb2*, *Atp9a*, *Atp7b*, *Timm10*, and *Nritp*, which are involved in ion transport. The active extrusion of xenobiotics by commonly shared transport proteins mainly located in the liver, kidney, and intestine as one of the important defense mechanisms. The genes involved in ion or solute transport activity might have significant implications in toxicology studies.

Albumin D-site binding protein (DBP) can activate the promoter of a putative clock oscillating gene, *Per1*, by directly binding to the Per1 promoter (Yamaguchi et al., 2000; Yan, Miyake, & Okamura, 2000). The *Per1* promoter is cooperatively activated by DBP and CLOCK-BMAL1. Contrarily, *Dbp* transcription is activated by CLOCK-BMAL1 through *E*-boxes and inhibited by the PER and CRY proteins, as is the case for *Per1*. Thus, *Dbp*, a clock-controlled gene whose expression oscillates with a very high circadian amplitude, might play an important role in the central clock oscillation. *Dbp* participates in the regulation of several clock outputs, including locomotor activity, sleep distribution, and liver gene expression. Furthermore, DBP is a major factor controlling the circadian expression of the steroid *15 α-hydroxylase* (*Cyp2a4*) and *coumarin 7-hydroxylase* (*Cyp2a5*) genes in mouse liver (Lavery et al., 1999).

A significant portion of the transcriptome in mammals, including the PAR-domain basic leucine zipper (PAR bZip) transcription factors DBP, HLF, and TEF, is under the circadian clock control. Triple mutant mice were born at the expected Mendelian ratios, but were epilepsy prone, aged at an accelerated rate, and died prematurely (Gachon, Olela, Schaad, Descombes, & Schibler, 2006). DBP, TEF, and HLF accumulate in a highly circadian manner in several peripheral tissues. To clarify the PAR bZip target genes, the liver and kidney transcriptomes of PAR bZip triple knockout mice were compared with those of wild-type or heterozygous mutant mice. The disruption of these three genes in

mice altered the gene expression patterns of several proteins involved in drug metabolism and in the liver and kidney responses to xenobiotic agents. The PAR bZip proteins control the expression of several enzymes and regulators involved in the detoxification and metabolism drugs, such as *Cyp* enzymes, carboxylesterases, aminolevulinic acid synthase, P450-oxidoreductase, sulfotransferases, GST, aldehyde dehydrogenases, UDP-glucuronosyltransferases, members of drug transporter families, and constitutive androstane receptor (CAR). Some genes encoding detoxification enzymes, such as CYP2A5, CYP2C50, and CES3, might be direct PAR bZip target genes. The expression of other detoxification enzymes, such as CYP2B10, is mostly controlled by the CAR, whose circadian transcription is governed by the PAR bZip proteins. Yet, other enzymes in the xenobiotic defense, such as aminolevulinic acid synthase and P450-oxidoreductase, appear to be under the control of both CAR and PAR bZip proteins. The various levels at which PAR bZip transcription factors might intervene in the coordination of xenobiotic detoxification have been schematically described (Fig. 4). Rhythmic changes in transcriptional regulators were analyzed, and the role of circadian clock in the xenobiotic detoxification system has been demonstrated.

Although the pharmacokinetics of several drugs that are mainly eliminated by the CYP3A4 metabolism vary according to their dosing time, the mechanism of the variation remains poorly understood. The 24-h oscillation in the expression of CYP3A4 mRNA is investigated in hepatic cells (Takiguchi et al., 2007). As brief exposure of HepG2 cells to 50% serum induced the 24 h oscillation in the expression of clock genes, serum-shocked HepG2 cells are employed as an in-vitro model to study the molecular mechanism underlying the circadian clock in the human liver. Both mRNA levels and metabolic activity of CYP3A4 in serum-shocked HepG2 cells fluctuate rhythmically with a period length of about 24 h. The oscillation in the expression of the CYP3A4 gene seems to be the underlying cause of the rhythmic change in its metabolic activity. Circadian transcriptional factor, D-site-binding protein (DBP), activates the transcription of the CYP3A4 gene by binding to the DNA sequence near the upstream of the transcriptional start site. The transactivation of the CYP3A4 gene by DBP is repressed by the E4 promoter-binding protein-4 (E4BP4), a negative component of the circadian clock. Consequently, DBP and E4BP4 consist of a reciprocating mechanism in which DBP activates the transcription of the CYP3A4 gene during the time of day when DBP is abundant, and E4BP4 suppresses the transcription at other times of day.

CYP2E1 is clinically and toxicologically important, but the enzymatic activity exhibits circadian oscillation in mouse liver (Matsunaga, Ikeda, Takiguchi, Koyanagi, & Ohdo, 2008). The expression of *CYP2E1* mRNA in mouse liver increases from the late light phase to the early dark phase. Luciferase reporter gene analysis reveals that hepatic nuclear factor-1alpha (HNF-1alpha) activates *CYP2E1* promoter activity, which is suppressed by CRY1. Repressor activity of CRY1 is observed on the HNF-1alpha binding site of the *CYP2E1* promoter region with mutated *E*-box. Treatment of 50% serum with HepG2 cells results in a 24-hour oscillation in the mRNA levels *CYP2E1*. Transfection of small interfering RNA against HNF-1alpha and CRY1 into serum-treated HepG2 cells attenuates the oscillation of *CYP2E1* mRNA expression. The results of the chromatin immunoprecipitation reimmunoprecipitation analysis reveals that, CRY1 time-dependently interacts with HNF-1alpha transcriptional complexes, including coactivator p300 on the HNF-1alpha binding site in the *CYP2E1* promoter. This time-dependent interaction causes circadian expression of CYP2E1 in hepatic cells.

Differentiated embryo chondrocyte-2 (DEC2), also known as *bHLHE41* or *Sharp1*, is a pleiotropic transcription repressor that controls the expression of genes involved in cellular differentiation, hypoxia responses, apoptosis, and circadian rhythm regulation (Matsunaga et al., 2012). DEC2 participates in the circadian control of hepatic metabolism by regulating the expression of CYP2D6, a major drug-metabolizing enzyme in humans. DEC2 interacts with CCAAT/enhancer-binding protein (C/EBPα), accompanied by formation of a complex with histone deacetylase-1, which suppresses the transcriptional activity of C/EBPα to induce the expression of *CYP2D6*. The oscillation in the protein levels of DEC2 in 50% serum-treated HepG2 cells is nearly antiphase to that in the mRNA levels of *CYP2D6*. Transfection of cells with small interfering RNA against DEC2 decreases the amplitude of *CYP2D6* mRNA oscillation in 50% serum-treated cells. DEC2 periodically represses the promoter activity of CYP2D6, resulting in its circadian expression in hepatic cells.

The expression of genes involved in xenobiotic detoxification is under the control of the circadian clock. The aryl hydrocarbon receptor

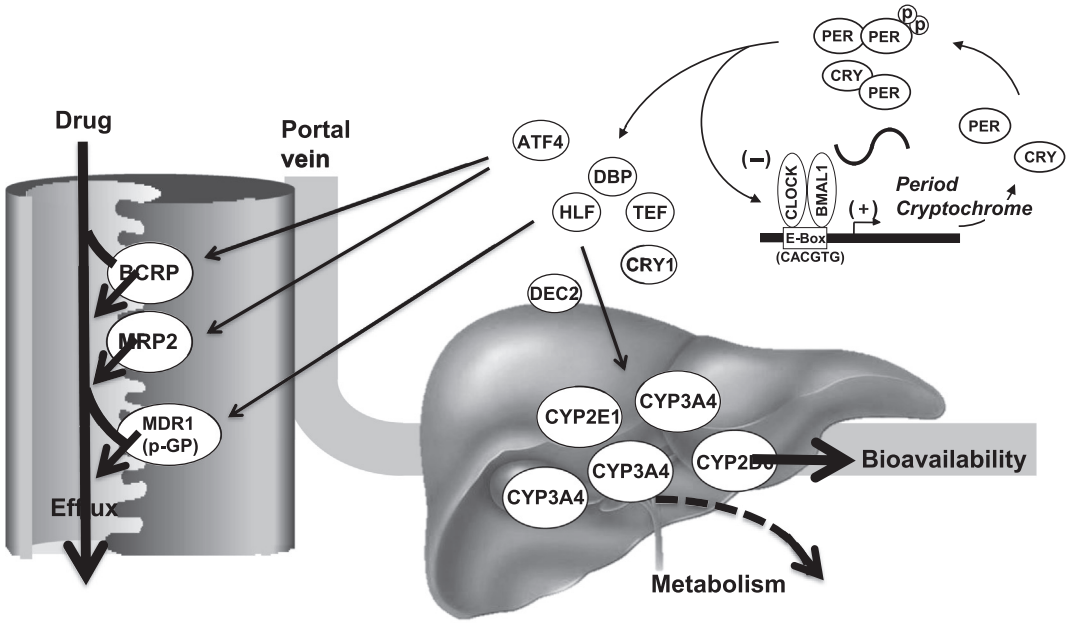

Fig. 4. Role of circadian clock in the xenobiotic detoxification and transporter system (Gachon et al., 2006). Model showing the different level of regulation of circadian detoxification by PAR-domain basic leucine zipper (PAR bZip) transcription factor. The PAR bZip transcription factors DBP, HLF, and TEF, is under circadian clock control. The PAR bZip proteins control the expression of many enzymes and regulators involved in drug metabolism and transport in intestine and liver. Some genes encoding detoxification enzymes and transporter may be direct PAR bZip target genes. The expression of other detoxification enzymes and transporter, is mostly controlled by CAR, whose circadian transcription is governed by PAR bZip proteins. Yet other enzymes and transporter in the xenobiotic defense appear to be under the control of both CAR and PAR bZip proteins.

(AhR) is one of the transcription factors responsible for the induction of detoxification enzymes in response to xenobiotic toxins, and the expression of AhR is regulated by molecular components of circadian clock (Tanimura, Kusunose, Matsunaga, Koyanagi, & Ohdo, 2011). The expression of AhR and its DNA binding ability in the lungs of mice exhibits circadian oscillation. Clock mutant (Clk/Clk) mice fail to show significant oscillation in the expression of AhR. The mRNA levels of AhR in the lungs of Clk/Clk mice are lower than in wild-type mice. A single intraperitoneal injection of benzo[α]pyrene, a ligand of AhR, induces the expression of *CYP1A1* in the lungs of wild-type mice, but the induction varies depending on the benzo[α]pyrene injection time. The dosing time-dependency of benzo[α]pyrene-induced *CYP1A1* expression is also modulated by Clock gene mutation. CLOCK protein affects the toxin-induced expression of detoxification enzymes through modulating the activity of AhR.

Xanthine oxidase (XOD), also known as xanthine dehydrogenase, is a rate-limiting enzyme in purine nucleotide degradation, which produces uric acid. Uric acid concentrations in the blood and liver exhibit circadian oscillations in both humans and rodents. The expression of XOD and its enzymatic activity exhibit circadian oscillations in the mouse liver (Kanemitsu et al., 2017). The orphan nuclear receptor peroxisome proliferator-activated receptor-α (PPARα) transcriptionally activates the mouse *XOD* gene and that bile acids suppresse *XOD* transactivation. The synthesis of bile acids is known to be under the control of the circadian clock, and the time-dependent accumulation of bile acids in hepatic cells interferes with the recruitment of the co-transcriptional activator p300 to PPARα, thereby repressing *XOD* expression. This time-dependent suppression of PPARα-mediated transactivation by bile acids causes an oscillation in the hepatic expression of XOD, which, in turn, leads to circadian alterations in uric acid production. The anti-hyperuricemic effect of the XOD inhibitor febuxostat is enhanced by administering it at the time of day before hepatic XOD activity increased.

P-glycoprotein (P-gp) is a ATP-binding cassette (ABC) transporters, which is encoded by *ABCB1* gene in human. In rodents, two members of P-gp have been identified—*Abcb1a* and *Abcb1b*—that probably fulfill the same functional role as P-gp in humans. P-gp functions as a xenobiotic transporter contributing to the intestinal barrier. The expression of the *Abcb1a* gene and efflux pump function of P-gp exhibit 24-h variation as described in Fig. 4. The molecular components of the circadian clock act as regulators to control 24-h variation in the expression of the *Abcb1b* gene (Murakami, Higashi, Matsunaga, Koyanagi, & Ohdo, 2008). The results of luciferase reporter assay and gel mobility shift assay reveal that hepatic leukemia factor (HLF) and E4 promoter binding protein-4 (E4BP4) regulate transcription of the *Abcb1a* gene by competing with each other for the same DNA binding site. These circadian transcriptional factors consist of a reciprocating mechanism in which HLF activates the transcription of the mdr1a gene, whereas E4BP4 periodically suppresses transcription at the time of day when E4BP4 is abundant. A significant 24-h variation in intestinal accumulation of digoxin is also observed in wild-type mice. The cyclic accumulation of digoxin is nearly antiphase to the rhythmicity of P-gp expression. On the other hand, Clock mutant mice fail to show significant 24-h variation in the intestinal accumulation of digoxin. Mean [^{3}H]-digoxin concentrations are consistently increased throughout the day, suggesting that efflux pump function of P-gp is reduced in Clock mutant mice.

Digested proteins are mainly absorbed as small peptides composed of two or three amino acids. The intestinal absorption of small peptides is mediated via only one transport system: the proton-coupled peptide transporter-1 (PepT1) encoded from the soluble carrier protein *SLC15A1*. In mouse, intestinal expression of PepT1/*Slc15a1* oscillates during the daily feeding cycle (Okamura et al., 2014). Although the oscillation in the intestinal expression of PepT1/*Slc15a1* is suggested to be controlled by molecular components of circadian clock, bile acids also participate in the circadian regulation of PepT1/*Slc15a1* expression through modulating the activity of PPARα. Nocturnally active mice mainly consume their food during the dark phase. PPARα activates the intestinal expression of *Slc15a1* mRNA during the light period, and protein levels of PepT1 peaks before the start of the dark phase. After food intake, bile acids accumulate in intestinal epithelial cells. Intestinal accumulated bile acids interfere with recruitment of co-transcriptional activator CREB-binding protein/p300 on the promoter region of *Slc15a1* gene, thereby suppressing PPARα-mediated transactivation of *Slc15a1*. The time-dependent suppression of PPARα-mediated transactivation by bile acids causes an oscillation in the intestinal expression of PepT1/*Slc15a1* during the daily feeding cycle that lead to circadian changes in the intestinal absorption of small peptides.

The data of microarray analysis have also identified *Slc22a4* gene, encoding organic cation transporter novel type-1 (OCTN1), as a PPARα-regulated gene and its intestinal expression exhibited circadian oscillations in a bile acid-dependent manner (Wada et al., 2015). In mouse small intestine, PPARα activates the expression of *Slc22a4* mRNA during the light period, and protein levels of OCTN1 peaks before the start of the dark phase. The bile acids that accumulate in intestinal epithelial cells suppress the PPARα-mediated transactivation of *Slc22a4* in the dark phase. The time-dependent suppression of PPARα-mediated transactivation by bile acids regulates oscillation in the intestinal expression of OCTN1/*Slc22a4* during the daily feeding cycle. The results of a pharmacokinetic analysis also reveals that oscillation in the expression of OCTN1 causes dosing time-dependent changes in the intestinal absorption of gabapentin and pregabalin. The dosing time-dependent changes in the bioavailability of these drugs affect their analgesics effects on neuropathic pain hypersensitivity (Akamine et al., 2015; Kusunose et al., 2010).

A number of diverse cell-surface proteins are anchored to the cytoskeleton via scaffold proteins. Na^+/H^+ exchanger regulatory factor-1 (NHERF1), encoded by the *Slc9a3r1* gene, functions as a scaffold protein, which is implicated in the regulation of membrane expression of various cell-surface proteins. The circadian clock component PERIOD2 (PER2) modulates transcription of the mouse *Slc9a3r1* gene, generating circadian accumulation of NHERF1 in the mouse liver (Tsurudome et al., 2018). Basal expression of *Slc9a3r1* is dependent on transcriptional activation by p65/p50. PER2 binds to p65 protein and prevents p65/p50-mediated transactivation of *Slc9a3r1*. The time-dependent interaction between PER2 and p65 underlies circadian oscillation in the hepatic expression of *Slc9a3r1*/NHERF1. The results of immunoprecipitation experiments and liquid chromatography-mass spectrometry analysis of mouse liver reveal that NHERF1 time-dependently interacts with fatty acid transport protein-5 (FATP5). Temporary accumulation of NHERF1 protein stabilizes plasmalemmal localization of FATP5, thereby enhancing hepatic uptake of fatty acids at certain times of the day. Because NHERF1 has been suggested to interact with a number of diverse cell-surface proteins, this scaffold protein may also be involved in the diurnal regulation of plasmalemmal localization of other plasma membrane proteins and their functions in hepatic cells.

From viewpoints of skin permeability associated with transport of water and glycerol, the biological function of skin exhibits a circadian rhythm. Aquaporin 3 (AQP3) is located in the basal layer of the epidermis and regulates biological functions of skin such as water content and trans-epidermal water loss. Mice mutated with *Clock* gene (Clk/Clk) decreases stratum corneum hydration. In the epidermis, the expression of mouse *Aqp3* exhibits circadian oscillation; but the oscillation is dampened in Clk/Clk mice (Matsunaga et al., 2014). The transcription of *mAqp3* gene is activated by DBP. The expression of a human homolog, *hAQP3*, also exhibits significant circadian oscillation in human keratinocyte (HaCaT) cells after synchronization with medium containing 50% serum, and this rhythm is regulated by the CLOCK/BMAL1 heterodimer. Although these data indicate that the molecular mechanisms underlying the rhythmic expression of *mAqp3* and *hAQP3* are different, clock genes are involved in the regulation of time-dependent skin hydration.

7. Chronopharmacological strategy

7.1. Therapeutic drug monitoring

The circadian stage-dependent changes in the kinetic aspects of drugs have been reported for several drugs as a function of the time of the day of administration. The intraindividual variability, such as circadian rhythm, and the interindividual variability become influencing factors in dosage adjustment. For example, the influence of dosing time on the accuracy in predicting plasma valproic acid (VPA) concentrations at the steady state has been investigated (Ohdo, Nakano, & Ogawa, 1990). Valproic acid 400 mg for nine days on a twice-daily basis (08:30 and 20:30 h) was administered to eight healthy male volunteers. The circadian changes in VPA kinetics occurred at the absorption phase. The prediction of VPA concentrations at 2 (around Cmax) and 12 h after both the morning and evening doses on day 9 was performed using individual subject's pharmacokinetic parameters and population pharmacokinetic parameters (Bayesian method) obtained from the morning trial on day 8. The predictive accuracy for VPA concentrations around the Cmax after the morning dose was better. However, the predictive accuracy for VPA concentrations after the evening dose was significantly biased towards overestimation. The individual pharmacokinetic parameters obtained from the morning trial were better sources for predicting the VPA concentrations around Cmax after the morning dose, but worse sources for that after the evening dose. The predictive performance based on Bayesian approach also showed findings similar to those based on individual pharmacokinetic parameters. The overestimation of the target points around Cmax after the evening dose was closely related to that of the absorption rate constant (Ka) value obtained using population parameters from the morning trial. Therefore, the time of the circadian stage at which VPA is administered is important for evaluating exactly the predictive accuracy.

The accuracy of predicting a single serum theophylline concentration using Bayesian method has been evaluated for an oral slow release form of theophylline using twice daily dosing (Chrystyn, Ellis, Mulley, & Peake, 1989). Further, 24-h steady state serum theophylline concentration-time profiles were obtained after the administration of one Uniphyllin Continus 400 mg tablet every 12 h in 15 patients. Theophylline absorption was faster during the day. When comparing the predicted and measured values, the accuracy of Bayesian method is considered more than adequate for clinical purposes. The predictions made using Bayesian method are statistically less biased and more precise than those derived using a theophylline algorithm using population mean data. The sample collected prior to a morning dose produced the most accurate predictions. No statistical difference was demonstrated for the predictive accuracy of samples drawn up to 4 h before or 2 h after the morning dose. All the serum theophylline concentrations to be used in Bayesian method should be drawn within this period. The predictive accuracy of drug concentration is improved when the time-of-day matched pharmacokinetic parameters are used. The intraindividual variability, such as the circadian rhythm, as well as interindividual variability should be considered in dosage adjustment.

7.2. Chrono-drug delivery system

Several studies have demonstrated the rationale behind chronotherapy (Altinok, Levi, & Goldbeter, 2009; FDA, 2003; Ohdo, Nakano, & Ogawa, 1991; Uematsu, Nakano, Kosuge, Kanamaru, & Nakashima, 1993). However, most studies on drug delivery over the past decades have focused on the constant drug release rate. The reason why the majority of DDS is designed with little emphasis on proven oscillatory phenomena might be in drug delivery limitations.

Chronopharmaceutics presents contemporary challenges to DDS. Due to the advances in chronobiology, chronopharmacology, and global market constraints, the traditional goal of pharmaceutics such as the constant drug release rate is becoming obsolete. However, a major bottleneck in the development of DDS that matches the circadian rhythm (Chrono-DDS) might be the availability of an appropriate technology. During recent years, we have witnessed the appearance of "Chrono-DDS" against various diseases. The high research interest in Chrono-DDS might lead to the emergence of chronopharmaceutics—a new sub-discipline in pharmaceutics. A principal objective of chronopharmaceutics is to introduce the drug at higher concentrations during the time of the greatest demand for the maximum therapeutic efficiency and at less concentrations when the need is less to minimize side effects.

The technologies in chronopharmaceutics includes: CONTIN® (physico–chemical modifications of the active pharmaceutical ingredient), OROS®, CODAS®, CEFORM®, DIFFUCAPS®, chronomodulating infusion pumps, TIMERx®, three-dimensional printing, controlled-release erodible polymer, and controlled-release microchip strategies (Alten, 2012; Ohdo, 2010; Ohdo et al., 2010; Youan, 2004) (Table 2). The following are some examples of Chrono-DDS available on the market: theophylline (Uniphyl®), famotidine (Pepcid®), simvastatin (Zocor®), COER-verapamil (Covera-HS®, Verelan® PM), diltiazem (Cardizem® LA), and propranolol (InnoPran® XL). Most data have been compiled from the Food and Drug Administration electronic orange book (FDA, Electronic, 2003), specific product package inserts, and the United States patents and specific pharmaceutical company websites. Future development in chronopharmaceutics might be made at the interface of other emerging disciplines such as system biology and nanomedicine. Such novel and more biological approaches to drug delivery might lead to safer and more efficient disease therapy in the future.

Modified-release (MR) prednisone is currently approved in the USA and several European countries for the treatment of adults with moderate-to-severe, active rheumatoid arthritis (RA), particularly when accompanied by morning stiffness (Alten, 2012). In patients with RA who switched from immediate-release (IR) to delayed-release (DR) prednisone, ($n = 110$), statistically significant reduction in morning stiffness occurred for over 3 months and were sustained for 9 months (Alten, Grahn, Holt, Rice, & Buttgereit, 2015). The absolute reduction in morning stiffness was for ~50 min with >40% relative reduction at each visit. The IL-6 level was reduced by the same amount. Statistically significant and clinically meaningful mean reductions in morning stiffness were maintained at >67 min at each visit along with significant improvements in pain and global assessment of the patients. There was no evidence of tachyphylaxis over the 9-month study. Patients receiving disease-modifying anti-rheumatic drugs (DMARDs) and IR prednisone who had not had significant reductions in morning stiffness demonstrated statistically significant and clinically meaningful improvements when switched to DR prednisone. Patients with symptomatic RA ($n = 350$) despite treatment with a DMARD were randomized 2:1 to receive additional therapy with DR prednisone 5 mg or placebo once daily for 12 weeks (Alten et al., 2015). Fatigue was assessed using the fatigue scale of the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) and the vitality domain of the Short Form-36 (SF-36). The general quality of life was assessed using the general score and individual domains of the Functional Assessment of Cancer Therapy-General and SF-36. Delayed release prednisone in addition to a DMARD significantly improved fatigue and other aspects of health-related quality of life in patients with symptomatic RA compared with that of DMARD treatment alone.

Tulobuterol was transdermally administered to healthy male volunteers (Uematsu et al., 1993). It was well absorbed after transdermal administration, with an absorption lag-time of approximately 4 h. The Cmax and area under the curve increased linearly with dose and the time-to-peak was approximately 9–12 h. The mean percent of drug absorbed during the application of a patch for 24 h was 82%–90% after a single dose and 82%–85% with repeated dosing. The mean urinary recovery as unchanged drug after a single inhalation and patch application was 3%–4% and 5%–6%, respectively. Tulobuterol does not accumulate during repeated inhalation or transdermal application. It is well tolerated, except for an increase in heart rate of 10–20 beats/min

Table 2
Examples of chrono-drug delivery system on the market in USA and Japan (Ohdo, 2010; Ohdo et al., 2010; Youan, 2010; Alten, 2012; Silas, Lakshmi, Ram, & Rao, 2017).

Active pharmaceutical ingredient (API)	Proprietary name dosages form	Proprietary chronopharmaceutical technology	Disease
	Other country		
Theophylline	Uniphyl® extended release tablets	CONTIN®	Asthma
Famotidine	Pepcid® tablets	Physico-chemical modification of API	Ulcer
Simvastatin	Zocor® tablets	Physico-chemical modification of API	Hyperlipideemia
Verapamil HCL	Covera-HS® extended release tablets	OROS®	Hypertension
Verapamil HCL	Verelan®PM extended release capsules	CODAS®	Hypertension
Diltiazem HCL	Cardizem® LA extended release tablets	CEFORM®	Hypertension
Propranolol HCL	InnoPran® XL extended release capsules	DIFFUCAPS®	Hypertension
Prednisone	LODOTRA®	MR (modified-release tablets)	Rheumatoid arthritis
Nifedipine	PROCARDIA® XL	TIMERx technology	Hypertension,amgina
Nifedipine	SOlfedipine® XL	TIMERx technology	Hypertension,amgina
Nifedipine	Cronodipin® XL	TIMERx technology	Hypertension
Oxybutynin	Crystrin®CR	TIMERx technology	Urinary incontinence
Oxymorphhone ER	Opana® ER	TIMERx technology	Pain
	Japan		
Famotidine	Gaster® tablets	Physico-chemical modification of API	Ulcer
Simvastatin	Lipovas® tablets	Physico-chemical modification of API	Hyperlipidemia
Theophylline	Uniphyl® extended release tablets	CONTIN®	Asthma
Tulobuterol	Hokunalin® Tape	Transdermal chrono-delivery system	Asthma

Theophylline is used for asthma associated with the increased bronchoconstriction in the early morning.
Famotidine is used for ulcer associated with the increased gastric acid secretion in the evening.
Simvastatin is used for hypercholesterolemia associated with the increased cholesterol synthesis in evening.
Verapamil, Diltiazem and Propranolol are used for hypertension associated with the increased blood pressure in morning.
Tulobuterol is used for asthma associated with the increased bronchoconstriction in the early morning.
Prednisone is used for the treatment of adults with moderate-to-severe, active rheumatoid arthritis (RA), particularly when accompanied by morning stiffness.

after five repeated applications of 4-mg patch. The patch with such an absorption pattern is useful for the chronotherapy of patients with nocturnal asthma.

Adriamycin-encapsulated liposomes modified with the Ala-Pro-Arg-Pro-Gly (APRPG) peptide (APRPG-LipADM) have been prepared, after it was shown that the APRPG peptide has affinity to angiogenic sites (Shimizu, Sawazaki, Tanaka, Asai, & Oku, 2008). Colon 26 NL-17 tumor-bearing mice were injected three times with APRPG-LipADM at zeitgeber times (ZTs) 2, 8, 14, and 20 where ZT 0 is the time when lights are turned on, and tumor growth was monitored. Tumor growth suppression changed with dosing time, and it was significantly more potent at ZT 14 than that at ZT 20. The circadian oscillation of VEGF is related to dosing time dependency with ANET. These results indicate that tumor growth suppression is correlated to some extent with the plasma VEGF concentration and that chronopharmacologic treatment of cancer with ANET might enhance the therapeutic efficacy and reduce the side effects.

The abundance of cell surface levels of transferrin receptor 1 (TfR1), which regulates the uptake of iron-bound transferrin, correlates with the rate of cell proliferation. Because the expression of TfR1 is higher in cancer cells than in normal cells, it offers a target for cancer therapy. Liposomes with ligands bonded to their external membrane surface have been studied in order to develop a method for delivering drugs or genes selectively to a target region (Okazaki et al., 2010). Transferrin N-glutaryl-dioleoyl-phosphatidylethanolamine (Tf-NGPE) liposomes were designed as intracellular targeting carriers of chemotherapeutic drugs into tumors. Okazaki et al. showed that the intratumoral delivery of a Pt-based anticancer drug, oxaliplatin (L-OHP), using Tf-NGPE liposomes varied depending on the 24-h variation in the expression of transferrin receptors on tumor cells in Colon 26 tumor-bearing mice. The growth of tumor cells implanted in mice was more severely inhibited by the intravenous injection of Tf-NGPE liposome encapsulated with L-OHP (7.5 mg/kg) in the early dark phase than when it was injected in the early light phase. The dosing-time dependency of anti-tumor effect was parallel to that of Pt incorporation into tumor DNA. Luciferase reporter analysis and chromatin immunoprecipitation experiments revealed that c-Myc regulated the 24-h oscillation of TfR1 expression in tumor cells, which underlay the dosing-time-dependent changes in the internalization of Tf-NGPE liposome-delivered L-OHP into the tumor cells. The intratumoral delivery of chemotherapeutic drug using Tf-NGPE liposomes is enhanced by administering the drug when the tumor expression of TfR1 is abundant. In the recent findings, Iron is an important biological catalyst and is critical for DNA synthesis during cell proliferation. Cellular iron uptake is enhanced in tumor cells to support DNA synthesis, but the synthesis varies in a circadian time-dependent manner (Okazaki et al., 2016). The levels of iron regulatory protein 2 (IRP2) exhibit circadian oscillation in colon-26 tumors implanted in mice. IRP2 regulates the expression of *Tfr1* mRNA at post-transcriptional level, by binding to RNA stem-loop structures known as iron-response elements. The transcription of *Irp2* mRNA is regulated by the components of circadian clock, BMAL1 and CLOCK heterodimer. The molecular circadian clock contributes to tumor cell proliferation by regulating iron metabolism.

8. Rhythm monitoring

The clock genes play a critical role in the molecular clockwork of the SCN and peripheral tissues such as the liver, kidney, and intestine. Communication between the SCN and peripheral tissues are likely to involve both nervous and humoral signals (Silver et al., 1996; Ueyama et al., 1999). Detection of the internal body time (BT) via a few-time-point assay has been a longstanding challenge in medicine, because BT information can be exploited to maximize potency and minimize toxicity during drug administration and thus will enable highly optimized medication.

A thorough understanding of the circadian clock requires a qualitative evaluation of circadian clock gene expression. No simple and effective method for detecting human clock gene expression is available. This limitation has greatly hampered our understanding of human circadian rhythm. A convenient, reliable, and less invasive method for detecting human clock gene expression using biopsy samples of hair follicle cells from the head or chin has been reported (Akashi et al., 2010). The circadian phase of clock gene expression in hair follicle cells accurately reflected that of individual behavioral rhythms, demonstrating that this strategy is appropriate for evaluating the human peripheral circadian clock. Furthermore, the method indicated that rotating shift workers suffer from a serious time lag between circadian gene expression rhythms and lifestyle. Qualitative evaluation of clock gene

expression in hair follicle cells, therefore, might be an effective approach for studying the human circadian clock in the clinical setting.

A convenient way to estimate internal BT is essential for chronotherapy and time-restricted feeding, both of which use body-time information to maximize potency and minimize toxicity during drug administration and feeding, respectively. To address this challenge, Minami et al. developed the concept, "molecular-timetable method," which was originally inspired by Linné's flower clock. With Linné's flower clock, one can estimate the time of the day by watching the opening and closing pattern of various flowers. Similarly, in the molecular-timetable method, one can measure the BT of the day by profiling the up and down patterns of substances in the molecular timetable. To make this method clinically feasible, Minami et al. (2009) performed blood metabolome analysis and reported the successful quantification of hundreds of clock-controlled metabolites in mouse plasma. Based on circadian blood metabolomics, they detected individual BT under various conditions, demonstrating its robustness against genetic background, sex, age, and feeding differences. This method was also demonstrated by the sensitive and accurate detection of circadian rhythm disorder in jet-lagged mice.

Furthermore, Minami et al. (2009) proposed a molecular timetable based on circadian-oscillating substances in multiple mouse organs or blood to estimate the BT from samples collected at only a few time points. Kasukawa et al. (2012) applied this molecular-timetable concept to estimate and evaluate the BT in human. They constructed a 1.5-d reference timetable of oscillating metabolites in human blood samples with a 2-h sampling frequency while simultaneously controlling the confounding effects of activity level, light, temperature, sleep, and food intake. By using this metabolite timetable as a reference, they accurately determined the internal BT within 3 h from just two anti-phase blood samples. Our minimally invasive, molecular-timetable method with human blood enables highly optimized and personalized medicine. These results suggest the potential for metabolomics-based detection of BT ("metabolite-timetable method"), which will lead to the realization of chronotherapy and personalized medicine.

9. Disruption and maintenance of biological rhythms

The alteration of circadian rhythm can be a side effect of several drugs (Terazono et al., 2008; Wollnik et al., 1995). The circadian clock system is necessary to adapt endogenous physiological functions to daily variations in environmental conditions. An abnormality in the circadian rhythms, such as the sleep–wake cycle and the timing of hormonal secretions, is implicated in various physiological and psychiatrical disorders. Recent molecular studies have revealed that oscillation in the transcription of specific clock genes plays a central role in the generation of 24-h cycles of physiology and behavior. It has been noticed that patients receiving chemotherapeutic agents experience disturbances in their behavioral and physical performances, including the circadian rhythms. It is important to evaluate the alteration of the circadian time structure, evidenced by delayed, advanced, or disrupted sleep–wake circadian rhythm, as a new side effect of medications that might show manifestation only as a result of particular administration-time schedule.

The associations between rest-activity circadian rhythm (CircAct) parameters, health-related quality of life (HRQoL), and survival have been shown in an independent cohort of chemotherapy-naïve patients with metastatic colorectal cancer participating in an international randomized phase III trial (Fig. 5) (Innominato et al., 2009). In a prior single-institution study, the extent of CircAct perturbation was independently predicted for survival and tumor response in 192 patients receiving chemotherapy for metastatic colorectal cancer. The main CircAct parameters correlated with several HRQoL scales. A prospective study attempted to extend these results to an independent cohort of chemotherapy-naïve patients with metastatic colorectal cancer participating in an international randomized phase III trial. The patients were randomized to receive chronomodulated or conventional infusion of 5-FU, leucovorin, and oxaliplatin as the first-line treatment for metastatic colorectal cancer. Patients from nine institutions completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-C30 and wore a wrist accelerometer (actigraph) for three days before chemotherapy delivery. Two validated parameters (I < O and r24) were used to estimate CircAct. Of the 130 patients with baseline CircAct assessments, 96 had baseline HRQoL data. I < O was confirmed to correlate with the global quality of life, physical functioning, social functioning, fatigue, and appetite loss. It further independently predicted the overall survival with a hazard ratio of 0.94. The associations between CircAct parameters, HRQoL, and survival, which were shown in this international study involving patients with previously untreated metastatic colorectal cancer, confirmed prior single-institution findings in mostly patients with pretreated metastatic colorectal cancer. The circadian timing system constitutes a novel therapeutic target. Interventions that normalize circadian timing system dysfunction might affect the quality of life and survival in patients with cancer.

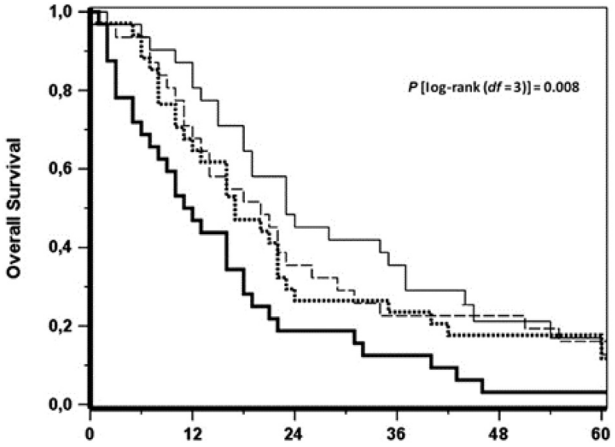

Fig. 5. Kaplan-Meier survival curves according to I < O split by the quartiles of their distribution (Reproduction with permission from Innominato et al., 2009). The rest-activity circadian rhythm (CircAct) parameters correlated with several health-related quality of life (HRQoL) scales. The circadian timing system constitutes a novel therapeutic target. Interventions that normalize circadian timing system dysfunction may affect quality of life and survival in cancer patients. The dichotomy index (I < O) integrates the circadian regulation of sleep and takes into account the relative difference in activity between the rest and wakeful spans. Thick solid line, first quartile; thin dashed line, second quartile; thick dotted line, third quartile; thin solid line, fourth quartile. Log-rank test (df = 3): $P = .008$ for I < O.

Shift work in human is probably carcinogenic associated with circadian rhythm disruption. Chronic jet-lag (CJL) suppresses the rhythms of behavior and physiology and accelerates the growth of two transplantable tumors in mice. The role of CJL as a tumor promoter has been investigated in mice exposed to the hepatic carcinogen, diethylnitrosamine (DEN) (Filipski et al., 2009). The mice that received DEN were randomized to remain in a photoperiodic regimen, where 12 h of light alternated with 12 h of darkness (LD 12:12) or subjected to CJL (8 h advance of light onset every two days). All the mice suffered from liver cancer caused by DEN. The diameter of the largest liver tumor was twice as large in CJL mice as compared with that in LD mice. LD mice presented a single histologic tumor type per liver. On the contrary, CJL mice presented up to four different types of tumor in the same liver. DEN disrupted the circadian rhythms of behavior and physiology in all mice. The association of circadian disruption with chronic DEN exposure suggests that the circadian clocks actively control the mechanisms of liver carcinogenesis in mice. Persistent circadian coordination might further be critical for slowing down and/or reverting cancer development after carcinogen exposure.

Interferons (IFNs) have been widely used as antiviral and antitumor agents. However, they cause adverse neuropsychiatric effects such as

depression and neurosis, which have been reported to sometimes lead to suicide (Fattovich, Giustina, Favarato, & Ruol, 1996; Janssen, Brouwer, van der Mast, & Schalm, 1994). When IFNs are administered during the early active phase in diurnally active humans, alterations in the 24-h rhythm are suggested by the changes in the lymphocyte count and cortisol level (Bocci, 1985a). There are significant and highly reproducible diurnal variations in several lymphocyte subsets with the lowest levels at 08:00–10:00 h and the highest at 22:00–24:00 h (Abo, Kawate, Itoh, & Kumagai, 1981; Bertouch, Roberts-Thomson, & Bradley, 1983; Haus, Lakatua, Swoyer, & Sackett-Lundeen, 1983; Ritchie et al., 1983). Cortisol remains at a low level during night, increases in the early morning, and reaches the maximum at around 08:00 h just at the time of the lowest levels of peripheral blood mononuclear cells (PBMCs). There is an inverse relationship between the plasma cortisol level and number of PBMCs. In most cases, IFNs have been administered in the morning associated with blood sampling (Bocci, 1985b). IFN administration causes a marked but transient rise in the total serum 11-hydroxycorticosteroid levels with a peak at around 8 h after injection (Scott et al., 1983). In other words, during IFN administration, particularly when administered daily, the cortisol level that normally decreases during the day will rise, and thus hindering the expected increase of PBMCs. IFN treatment inhibits the egress of lymphocytes from enlarged nodes and causes lymphopenia (Gresser, Guy-Grand, Maury, & Maunoury, 1981; Scott et al., 1981). Irrespective of the mechanism (Gallatin, Weissman, & Butcher, 2006), IFN administered in the morning seems to abrogate the normal recirculation of lymphocytes from tissues to blood and vice versa. The fact that immune processes to identify and eliminate tumor, allogenic, and virus-infected cells can be more active at a certain time of the day (between 20:00 and 02:00 h) is supported by the observation that the responses of PBMCs to mitogens and antigens varied inversely with the level of plasma cortisol (Cove-Smith, Kabler, Pownall, & Knapp, 1978; Escola et al., 1976) Therefore, a shift of about 12 h in IFN administration, from the morning to the late evening, can, in theory, render the drug more effective, and also result in less side effects to the patient.

Interferon-α (IFN-α) alters the rhythm of *Per* gene mRNA expression in the SCN (Ohdo et al., 2001). These findings are supported by the inhibitory effect of IFN-α on the mRNA expression of Clock and Bmal1. Furthermore, the rhythmicity of locomotor activity and body temperature are severely blunted by the repetitive administration of IFN-α. IFN-α acts on the SCN as evidenced by the expression of ISGF (Ohdo et al., 2001) and the rhythmicity that SCN control in the peripheral tissue. The rhythmicity of locomotor activity is severely altered by the continuous administration of corticosterone or a time-restricted feeding schedule while leaving the rhythmic phase of clock genes in the SCN unaffected (Boulos & Terman, 1980; Damiola et al., 2000). Thus, the possibility that altered locomotor activity could in turn lead to changed clock gene expression in the SCN is low in the case of IFN-α. The photic induction of the *Per1* gene in SCN is also completely disturbed by the daily administration of IFN-α at the early active phase, which may have caused a functional disorder in the resetting and entrainment of SCN. IFN-α sometimes causes ocular adverse effects associated with retinal or optic neuropathy (Lohmann, Kroher, Bogenrieder, Spiegel, & Preuner, 1999; Purvin, 1995), although the mechanism is not clear.

Interestingly, inhibitory effect on the mRNA expression of each clock gene in the SCN has been observed by the repetitive administration of IFN-α during the early active phase, but not the early rest phase (Ohdo et al., 2001). A similar dosing schedule-dependent inhibition of Per1 mRNA expression has been demonstrated during the repetitive administration of IFN-γ, which can be induced by IFN-α or IFN-β in combination with other cytokines (Hunter, Gabriel, Radzanowski, Neyer, & Remington, 1997). The expression of IFN-γ receptor in SCN follows a 24-h rhythm with a peak at the early active phase (Lundkvist, Robertson, Mhlanga, Rottenberg, & Kristensson, 1998). This might be why the administration of IFN-α during the early rest phase can reduce its side effect. The observations described above in human correspond well to the findings indicating that the alteration of clock gene expression is induced by IFN-α administration during the early active phase in nocturnally active rodents. Also, the influence of 5-FU on the expression of clock genes has been investigated to explore the underlying mechanism of chemotherapeutic agent-induced disturbance of these rhythms (Terazono et al., 2008). Continuous administration of 5-FU to mice attenuated the oscillation in the expressions of Per1 and Per2 mRNA in the liver and SCN. These results reveal a possible pharmacological action by 5-FU on the circadian clock mechanism, which might be the underlying cause of its adverse effects on the 24-h rhythms of physiology and behavior.

10. Adjustment and manipulation of biological rhythms

The 24-h rhythms of physiology and behavior are influenced by various environmental factors such as feeding schedules, genetic factors, and social interactions, as well as lighting condition and several drugs (Akiyama et al., 1999; Aschoff, 1963; Boulos & Terman, 1980; Horikawa et al., 2000). As the period of the central circadian pacemaker in human is slightly longer than 24 h, synchronization of the circadian system with the light–dark cycle occurs by daily phase-advances of the circadian clock. In human, the time-of-day dependent phase-shifting effects of light have been summarized in a phase-response curve (Duncan Jr., 1996). Morning light advances the central circadian pacemaker, late afternoon and evening light delays the pacemaker, and light during the midday is without phase-shifting effects. On the contrary, the phase-shifting agents such as melatonin, and 5-HT, and behavioral arousal have a phase-response curve distinct from that of light. The phase advances occur between midday and early evening. The phase delays occur between late night and midday. Phase shifts produced by nonphotic zeitgebers are similar to phase shifts produced by dark pulses presented to animals housed under constant light. The extrinsic timekeeping such as photic and nonphotic effects on intrinsic timekeeping might be important components of disordered timekeeping in depressive illness.

The SCN neurons receive information about light intensity in the environment via direct synaptic connections with the retina, which adapts the phase of SCN oscillator to the photoperiod. The SCN clock then synchronizes overt rhythms in physiology and behavior. Per1 and Per2 transcription is rapidly induced by light in a time-of-day-dependent manner (Shigeyoshi et al., 1997). The responsiveness of Per1 mRNA to light is closely related to behavioral phase delays induced by light. Light-induced phase delays in locomotor activity during subjective night are significantly inhibited when mice are pretreated with Per1 antisense phosphorothioate oligodeoxynucleotide (Akiyama et al., 1999). Therefore, the gated expression of Per1 might be an important step in causing photic entrainment.

It is well known that not only photic but also nonphotic stimuli can entrain the SCN clock, and several drugs have been investigated to modulate the circadian rhythm by causing a phase shift in the rhythm in the peripheral or central nervous system (Duncan Jr., 1996). The acute and circadian time-dependent reduction in Per1 and/or Per2 mRNA in the hamster SCN by 5-HT1A/7 receptor agonists strongly correlated with phase resetting in response to the drug (Horikawa et al., 2000). Therefore, nonphotic shifts might involve change in the Per1 and/or Per2 mRNA levels in the SCN.

A variety of physiological rhythmic variables are influenced by the cyclic variation of environmental factors (Aschoff, 1963). One of those factors is the feeding schedule (Boulos & Terman, 1980). A time-restricted feeding schedule can change the rhythmic phase of locomotor activity and physiological function including the corticosterone level. Such effects are not influenced by the SCN lesions (Krieger, Hauser, & Krey, 1977). Ventromedial hypothalamic lesions abolish food-shifted circadian adrenal rhythmicity (Krieger, 1980). The paraventricular nucleus appears to be the site where feeding-associated circadian

oscillation is connected to the HPA axis (Honma, Noe, Honma, Katsuno, & Hiroshige, 1992).

In human, the pattern of diet intake substantially modifies the plasma cortisol levels and body temperature rhythm (Nishimura, Kato, & Saito, 1992; Saito, Nishimura, & Kato, 1989). Specifically, the rhythmicity of the plasma cortisol levels can be maintained normal only when the feeding pattern is diurnal, but is reversed or disturbed under a nocturnal or continuous feeding pattern. To clarify the relationship between the pattern of diet intake and the circadian adrenocortical rhythm, the plasma cortisol levels and body temperature were measured at 4-h intervals over a 24-h period in 18 patients who were in the vegetative state and had been receiving total enteral nutrition (TEN) for four weeks. One group of patients was provided a liquid diet intraduodenally and continuously throughout a day (continuous TEN), whereas two other groups received their daily enteral feeding during a restricted time of the day, either in the daytime from 08:00 to 20:00 h (diurnal TEN, 6 patients) or in the nighttime from 20:00 to 08:00 h (nocturnal TEN, 6 patients). In patients with diurnal TEN, there was a clear cortisol rhythm with a peak of 08:00 h, whose pattern was quite similar to the well-established cortisol rhythm in normal subjects. Patients with nocturnal TEN also showed a cortisol rhythm, but the peak appeared at 16:00 h. There was no appreciable difference in the amplitude of the rhythm between the two groups. Patients with continuous TEN did not show any consistent circadian cortisol rhythms. In the diurnal TEN group, there was a clear body temperature rhythm with a peak at 20:00 h, whose pattern was similar to the well-established body temperature rhythm in normal subjects. The nocturnal TEN group also showed a temperature rhythm, but the peak appeared at 04:00 h. The continuous TEN group did not show any consistent body temperature rhythms. These results suggest that the timing of diet intake may have a synchronizing effect on the circadian cortisol rhythm in human.

The manipulation of feeding schedule can modify the chronopharmacological action and chronopharmacokinetics of a drug and the rhythmicity of molecular clock (Damiola et al., 2000; Ohdo et al., 1996). Circadian synchronization of cell proliferation is observed not only in normal healthy tissues but also in malignant solid tumors. However, DNA synthesis in tumor cells implanted in mice showed a 24-h oscillation apparently differing from that of normal bone marrow cells. The peculiar rhythm of tumor cell proliferation is modulated by the inhibition of platelet-derived growth factor (PDGF) signaling (Nakagawa et al., 2006; Ohdo, 2010; Ohdo et al., 2010). Continuous administration of AG1295 (10 μg/h, s.c.), a PDGF receptor tyrosine kinase inhibitor, substantially suppressed DNA synthesis in the implanted tumor cells, but not in healthy bone marrow cells. During the administration of this drug, the rhythm of DNA synthesis in the tumor cells is synchronized with that in bone marrow cells. To produce new rhythmicity by manipulating the conditions of organs using rhythmic administration of altered feeding schedules or several drugs appears to lead to a new concept in chronopharmacotherapy.

11. Chrono-drug discovery and development

Clock genes are expressed not only in the SCN, but also in other brain regions and various peripheral tissues. Such a cascade of clock genes may contribute to the organization of biological rhythms in the whole body. An example of circadian transcription factors in molecular target, metabolic enzyme and transporter of antitumor drug is shown in Table 3. Not only the rhythm in transcription, but also the rhythm in post-transcription, protein degradation and genome editing contribute to the rhythmicity of molecular target. Other examples for chrono-drug discovery are described below.

Malignant phenotypes of triple-negative breast cancer (TNBC) are subject to circadian alterations, but the role of cancer stem cells (CSC) in defining this circadian change has not been understood. CSC are often characterized by high aldehyde dehydrogenase (ALDH) activity, which is associated with the malignancy of cancer cells and is used for identification and isolation of CSC. The population of ALDH-positive cells in a mouse 4 T1 breast tumor model exhibits pronounced circadian alterations (Matsunaga et al., 2018). Alterations in the number of ALDH-positive cells are generated by time-dependent increases and decreases in the expression of *Aldh3a1* gene. Importantly, circadian clock genes are rhythmically expressed in ALDH-negative cells, but not in ALDH-positive cells. Circadian expression of *Aldh3a1* in ALDH-positive cells is dependent on the time-dependent release of Wingless-type mmtv integration site family 10a (WNT10a) from ALDH-negative cells. Furthermore, antitumor and antimetastatic effects of ALDH inhibitor N, N-diethylaminobenzaldehyde are enhanced by administration at the time of day when ALDH activity is increased in 4T1 tumor cells. These data reveal a new role for the circadian clock within the tumor microenvironment in regulating the circadian dynamics of CSC and may also

Table 3
Circadian transcription factors in molecular target, metabolic enzyme and transporter of antitumor drug.

Gene name	Positive transcriptional regulator	Negative transcriptional regulator	DNA binding site	Reference
Irp2	CLOCK,BMAL1	CRY1,PER2	*E*-box	Okazaki et al. (2016)
Fbxw7	DBP	E4BP4	D-site	Okazaki et al. (2014)
Drd3	RORα	Nr1d1	RORE	Ikeda et al. (2013)
5-htt	ATF4	–	ATF4 binding site	Ushijima et al. (2012)
Tfr1	c-Myc	–	E-box	Okazaki et al. (2010)
Pai-1	PPARα/RARα	–	PPRE	Oishi et al. (2010)
	CLOCK/BMAL1	CRY1,PER2	E-box	Hayashida et al. (2010)
MetAp2	CLOCK/BMAL1	CRY1,PER2	E-box	Nakagawa et al. (2004)
Vegf	HIF1	PER2	HRE	Koyanagi et al. (2003)
Mpg	CLOCK,BMAL1	DEC1	E-box	Kim, Matsunaga, Koyanagi, and Ohdo (2009)
Mgmt	HLF	E4BP4	D-site	Horiguchi et al. (2010)
CYP2D6	C/EBPα/HNF-4α	DEC2	C/EBPα-binding site	Matsunaga et al. (2012)
CYP1A1	CLOCK	CRY1,PER2	E-box	Tanimura et al. (2011)
CYP2E1	HNF-1α	CRY1	HNF-1 binding site	Matsunaga et al. (2008)
CYP3A4	DBP	E4BP4	D-site	Takiguchi et al. (2007)
Slc9a3r1	p65/p50	PER2	NRE	Tsurudome et al. (2018)
Slc22a2	PPARα/RARα	–	PPRE	Oda et al. (2014)
Slc15a1	PPARα/RARα	Cholic acid	PPRE	Okamura et al. (2014)
Slc22a4	PPARα/RARα	Cholic acid	PPRE	Wada et al. (2015)
hAqp3	CLOCK,BMAL1	CRY1,PER2	E-box	Matsunaga et al. (2014)
mAqp3	DBP	E4BP4	D-site	Matsunaga et al. (2014)
Abcg2	ATF4	–	ATF4 binding site	Hamdan et al. (2012)
Abcb1a	HLF	E4BP4	D-site	Murakami et al. (2008)

lead to the development of novel therapeutic strategies for treatment of TNBC with ALDH inhibitors.

Chronic kidney disease (CKD) is a global health problem, and novel therapies to treat CKD are urgently needed. Renal expression of chemokine (C—C motif) ligand 2 (CCL2) is increased in response to p65 activation in the kidneys of 5/6-nephrectomized (5/6Nx) mice. G0 s2 plays an important role for p65-induced transactivation of m*Ccl2* in 5/6Nx mice (Matsunaga et al., 2016). These pathologies in the kidney of 5/6Nx mice are ameliorated by down-regulation of G0 s2. Furthermore, a novel small-molecule inhibitor against G0 s2 expression is identified by high-throughput chemical screening, and the inhibitor suppresses renal inflammation in 5/6Nx mice. Circadian clocks are molecular time-keeping systems that underlie daily fluctuations in multiple physiological and biochemical processes. The dysfunctions of the circadian system are associated with the development of various pathological conditions. The application of high throughput screening approach is performed to search for small molecules capable of pharmacological modulation of the molecular clock (Matsunaga et al., 2016). The evidence for the feasibility and value of this approach is being accumulated for both scientific and therapeutic purposes.

CKD is often exacerbated with an increase in serum retinol; however, the reason has not been elucidated yet. The liver is the major organ responsible for retinol metabolism; the expressions of *Cyp3a11* and *Cyp26a1*, encoding key enzymes of retinol metabolism, are reduced in 5/6Nx mice (Hamamura et al., 2016; Matsunaga et al., 2016) (Fig. 6). The reduction is mediated by the decreased expression of DBP. Furthermore, an increase in plasma transforming growth factor-β1 (TGF-β1) in 5/6Nx mice leads to the decreased expression of the *Dbp* gene. Consistently, the alterations of retinol metabolism and renal dysfunction in 5/6Nx mice are ameliorated by administration of an anti-TGF-β1 antibodies. The accumulation of serum retinol induces the apoptosis of renal cells of 5/6Nx mice fed with a normal diet, whereas renal dysfunction was attenuated in mice fed with a retinol-free diet. These findings indicate that constitutive reduction of DBP expression induces the disruption of retinol metabolism in hepatic cells under CKD condition. Thus, the aggravation of renal dysfunction in patients with CKD might be prevented by a recovery of hepatic function, potentially through therapies targeting DBP and retinol.

Recent studies in laboratory rodents have revealed that circadian oscillation in the physiologic functions affecting drug disposition underlies the dosing time-dependent change in pharmacokinetics. However, it is difficult to predict the circadian change in the drug pharmacokinetics in a diurnal human by using the data collected from nocturnal rodents. Cynomolgus monkeys, diurnal active animals, are evaluated the relevance of intestinal expression of P-glycoprotein (P-gp) to the dosing time dependency of the pharmacokinetics of its substrates (Iwasaki et al., 2015). The rhythmic phases of circadian gene expression in the SCN of cynomolgus monkeys are similar to those reported in nocturnal rodents. On the other hand, the expression of circadian clock genes in the intestinal epithelial cells of monkeys oscillates at opposite phases in rodents. The intestinal expression of P-gp in the small intestine of monkeys is also oscillated in a circadian time-dependent manner. Furthermore, the intestinal absorption of P-gp substrates (quinidine and etoposide) is substantially suppressed by administering the drugs at the times of day when P-gp levels are abundant. By contrast, there is no significant dosing time-dependent difference in the absorption of the non-P-gp substrate (acetaminophen). The oscillation in the intestinal expression of P-gp appears to affect the pharmacokinetics of its substrates. Identification of circadian factors affecting the drug disposition in laboratory monkeys may improve the predictive accuracy of pharmacokinetics in humans.

12. Conclusions

An understanding of 24-h rhythm in the risk of disease plus evidence of 24-h of rhythm dependency of drug PK, effects, and safety constitutes the rationale for pharmacotherapy. The drugs for several diseases are still administered without regard to the time of the day. The chronopharmacological findings should be systematically summarized in an applicable format for clinical practice. A reference rhythm for circadian timing of medications plays a key role to achieve the purpose. Identification of a rhythmic marker for selecting dosing time can lead to improved

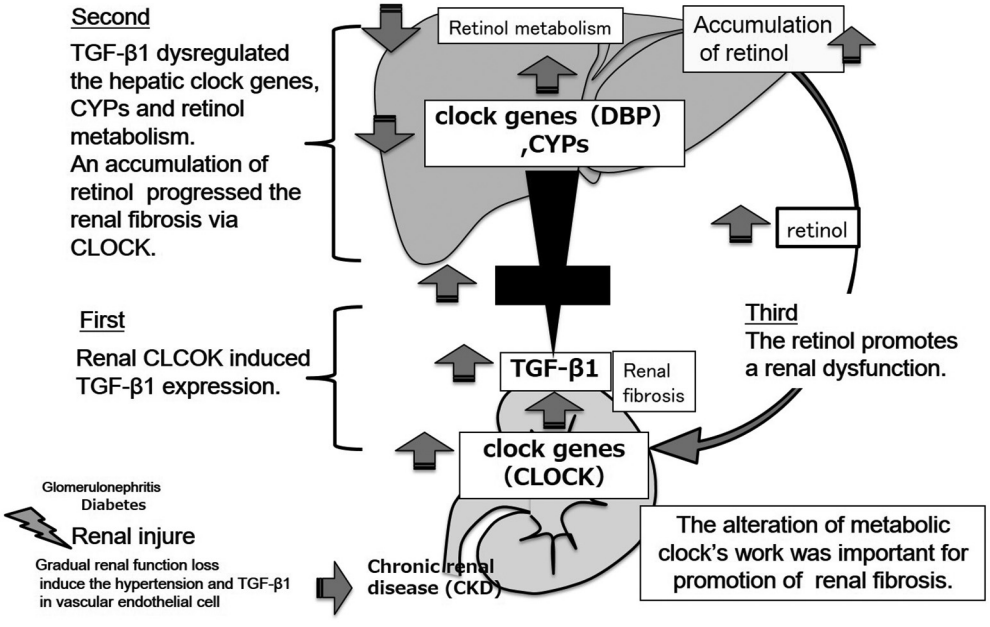

Fig. 6. The novel mechanism of progression in chronic kidney disease (CKD) based on the alteration of metabolic clock (Hamamura et al., 2016; Matsunaga et al., 2016). CKD is associated with an increase in serum retinol. The alteration of hepatic metabolism induced the accumulation of serum retinol in 5/6 nephrectomy (5/6Nx) mice. D-box-binding protein (DBP), which controls the expression of several CYP genes, was significantly decreased in these mice. Cyp3a11 and Cyp26a1, encoding key proteins in retinol metabolism, showed the greatest decrease in expression in 5/6Nx mice, a process mediated by the decreased expression of DBP. Furthermore, an increase of plasma transforming growth factor-β1 (TGF-β1) in 5/6Nx mice led to the decreased expression of the Dbp gene. The accumulation of serum retinol induced renal apoptosis in 5/6Nx mice fed a normal diet, whereas renal dysfunction was reduced in mice fed a retinol-free diet. Thus, the aggravation of renal dysfunction in patients with CKD might be prevented by a recovery of hepatic function, potentially through therapies targeting DBP and retinol.

progress and diffusion of chronopharmacotherapy. To monitor the rhythmic markers such as clock genes, it might be useful to choose the most appropriate time of the day for the administration of drugs that might increase their therapeutic effects and/or reduce their side effects. Furthermore, to produce new rhythmicity by manipulating the conditions of organs by rhythmic administration of drugs at altered feeding schedules appears to lead to a new concept in chronopharmacotherapy. Attention should be paid to the alteration of biological rhythm and consider it an adverse effect when it leads to altered regulation of the circadian system, which is a serious problem affecting the basic functioning of living organisms. An approach to increase the efficiency of pharmacotherapy is administering drugs at times when they are best tolerated.

The clock genes ultimately control a vast array of circadian rhythms in physiology and behavior. They regulate several diseases such as cancer, metabolic syndrome, and sleep. The monitoring of rhythm, overcoming rhythm disruption, and manipulating rhythm from the viewpoint of molecular clock is essential to improve the progress and diffusion of chronopharmacotherapy. Chrono-DDS might aid in the development of new therapeutic strategies for several diseases and provide insights into chronotherapy as a way to optimize current therapies. Recent studies on pharmacotherapy have focused on gene delivery and antibody delivery targeting specific molecular for some diseases. The clock genes should also be one of the important candidates. Several academic laboratories are screening for small molecules targeting the circadian clock to stabilize its phase and enhance its amplitude, and thereby consolidate and coordinate circadian organization, which in turn is likely to help prevent and control several diseases including sleep disorder and cancer etc. in human. These drugs and strategies can be used to make patients with several diseases feel and function more normally. Such a strategy might be applicable to target clock genes in chronotherapy. Further elucidating the connections between clock genes, and PK and PD could benefit the development of new therapeutic strategies for several diseases and provide insights into chronotherapy as an approach to optimize current therapies.

Conflict of interest statement

The authors have no competing interests to declare.

Acknowledgments

This research was supported by Platform Project for Supporting Drug Discovery and Life Science Research [Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)] from AMED under Grant Number JP18am0101091.

References

Abo, T., Kawate, T., Itoh, K., & Kumagai, K. (1981). Studies on the bioperiodicity of the immune response. I. Circadian rhythms of human T, B, and K cell traffic in the peripheral blood. *Journal of Immunology 126*, 1360–1363.

Akamine, T., Koyanagi, S., Kusunose, N., Hashimoto, H., Taniguchi, M., Matsunaga, N., & Ohdo, S. (2015). Dosing time-dependent changes in the analgesic effect of pregabalin on diabetic neuropathy in mice. *The Journal of Pharmacology and Experimental Therapeutics 354*, 65–72.

Akashi, M., Soma, H., Yamamoto, T., Tsugitomi, A., Yamashita, S., Yamamoto, T., ... Node, K. (2010). Noninvasive method for assessing the human circadian clock using hair follicle cells. *Proceedings of the National Academy of Sciences of the United States of America 107*, 15643–15648.

Akiyama, M., Kouzu, Y., Takahashi, S., Wakamatsu, H., Moriya, T., Maetani, M., ... Shibata, S. (1999). Inhibition of light- or glutamate-induced mPer1 expression represses the phase shifts into the mouse circadian locomotor and suprachiasmatic firing rhythms. *The Journal of Neuroscience 19*, 1115–1121.

Alten, R. (2012). Chronotherapy with modified-release prednisone in patients with rheumatoid arthritis. *Expert Review of Clinical Immunology 8*, 123–133.

Alten, R., Grahn, A., Holt, R. J., Rice, P., & Buttgereit, F. (2015). Delayed-release prednisone improves fatigue and health-related quality of life: Findings from the CAPRA-2 double-blind randomised study in rheumatoid arthritis. *RMD Open 1*, e000134.

Alten, R., Holt, R., Grahn, A., Rice, P., Kent, J., Buttgereit, F., & Gibofsky, A. (2015). Morning stiffness response with delayed-release prednisone after ineffective course of immediate-release prednisone. *Scandinavian Journal of Rheumatology 44*, 354–358.

Altinok, A., Levi, F., & Goldbeter, A. (2009). Identifying mechanisms of chronotolerance and chronoefficacy for the anticancer drugs 5-fluorouracil and oxaliplatin by computational modeling. *European Journal of Pharmaceutical Sciences 36*, 20–38.

Aschoff, J. (1963). Comparative physiology: Diurnal rhythms. *Annual Review of Physiology 25*, 581–600.

Ayalon, L., Hermesh, H., & Dagan, Y. (2002). Case study of circadian rhythm sleep disorder following haloperidol treatment: Reversal by risperidone and melatonin. *Chronobiology International 19*, 947–959.

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., ... Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science 289*, 2344–2347.

Beato, M., Herrlich, P., & Schutz, G. (1995). Steroid hormone receptors: Many actors in search of a plot. *Cell 83*, 851–857.

Bertouch, J. V., Roberts-Thomson, P. J., & Bradley, J. (1983). Diurnal variation of lymphocyte subsets identified by monoclonal antibodies. *British Medical Journal (Clinical Research Ed.) 286*, 1171–1172.

Bocci, V. (1985a). Administration of interferon at night may increase its therapeutic index. *Cancer Drug Delivery 2*, 313–318.

Bocci, V. (1985b). In I. N. B. Finter, & R. Oldham (Eds.), *Distribution catabolism and pharmacokinetics of interferons* (pp. 47–72). Amsterdam: Elsevier.

Boulos, Z., & Terman, M. (1980). Food availability and daily biological rhythms. *Neuroscience and Biobehavioral Reviews 4*, 119–131.

Ceresa, F., Angeli, A., Boccuzzi, G., & Molino, G. (1969). Once-a-day neurally stimulated and basal ACTH secretion phases in man and their response to corticoid inhibition. *The Journal of Clinical Endocrinology and Metabolism 29*, 1074–1082.

Chang, D. C., & Reppert, S. M. (2001). The circadian clocks of mice and men. *Neuron 29*, 555–558.

Chrystyn, H., Ellis, J. W., Mulley, B. A., & Peake, M. D. (1989). Bayesian derived predictions for twice daily theophylline under outpatient conditions and an assessment of optimal sampling times. *British Journal of Clinical Pharmacology 27*, 215–221.

Cove-Smith, J. R., Kabler, P., Pownall, R., & Knapp, M. S. (1978). Circadian variation in an immune response in man. *British Medical Journal 2*, 253–254.

Dagan, Y. (2002). Circadian rhythm sleep disorders (CRSD) in psychiatry—A review. *The Israel Journal of Psychiatry and Related Sciences 39*, 19–27.

Dagan, Y., Yovel, I., Hallis, D., Eisenstein, M., & Raichik, I. (1998). Evaluating the role of melatonin in the long-term treatment of delayed sleep phase syndrome (DSPS). *Chronobiology International 15*, 181–190.

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 & Development 14*, 2950–2961.

Desai, V. G., Moland, C. L., Branham, W. S., Delongchamp, R. R., Fang, H., Duffy, P. H., ... Fuscoe, J. C. (2004). Changes in expression level of genes as a function of time of day in the liver of rats. *Mutation Research 549*, 115–129.

Duncan, W. C., Jr. (1996). Circadian rhythms and the pharmacology of affective illness. *Pharmacology & Therapeutics 71*, 253–312.

Fattovich, G., Giustina, G., Favarato, S., & Ruol, A. (1996). A survey of adverse events in 11,241 patients with chronic viral hepatitis treated with alfa interferon. *Journal of Hepatology 24*, 38–47.

FDA (2003). *Electronic orange book*. Washington, DC: Administration, F.a. D.

Filipski, E., Subramanian, P., Carriere, J., Guettier, C., Barbason, H., & Levi, F. (2009). Circadian disruption accelerates liver carcinogenesis in mice. *Mutation Research 680*, 95–105.

Gachon, F., Olela, F. F., 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 Metabolism 4*, 25–36.

Gallatin, W. M., Weissman, I. L., & Butcher, E. C. (2006). A cell-surface molecule involved in organ-specific homing of lymphocytes. *J. Immunol. 177*, 5–9.

Gresser, I., Guy-Grand, D., Maury, C., & Maunoury, M. T. (1981). Interferon induces peripheral lymphadenopathy in mice. *Journal of Immunology 127*, 1569–1575.

Halberg, F. (1969). Chronobiology. *Annual Review of Physiology 31*, 675–725.

Hamamura, K., Matsunaga, N., Ikeda, E., Kondo, H., Ikeyama, H., Tokushige, K., ... Ohdo, S. (2016). Alterations of hepatic metabolism in chronic kidney disease via D-box-binding protein aggravate the renal dysfunction. *The Journal of Biological Chemistry 291*, 4913–4927.

Hamdan, A. M., Koyanagi, S., Wada, E., Kusunose, N., Murakami, Y., Matsunaga, N., & Ohdo, S. (2012). Intestinal expression of mouse Abcg2/breast cancer resistance protein (BCRP) gene is under control of circadian clock-activating transcription factor-4 pathway. *The Journal of Biological Chemistry 287*, 17224–17231.

Haus, E., Lakatua, D. J., Swoyer, J., & Sackett-Lundeen, L. (1983). Chronobiology in hematology and immunology. *The American Journal of Anatomy 168*, 467–517.

Hayashida, S., Kuramoto, Y., Koyanagi, S., Oishi, K., Fujiki, J., Matsunaga, N., ... Soeda, S. (2010). Proxisome proliferator-activated receptor-alpha mediates high-fat, diet-enhanced daily oscillation of plasminogen activator inhibitor-1 activity in mice. *Chronobiology International 27*, 1735–1753.

Hermesh, H., Lemberg, H., Abadi, J., & Dagan, Y. (2001). Circadian rhythm sleep disorders as a possible side effect of fluvoxamine. *CNS Spectrums 6*, 511–513.

Honma, K., Noe, Y., Honma, S., Katsuno, Y., & Hiroshige, T. (1992). Roles of paraventricular catecholamines in feeding-associated corticosterone rhythm in rats. *The American Journal of Physiology 262*, E948–E955.

Horiguchi, M., Kim, J., Matsunaga, N., Kaji, H., Egawa, T., Makino, K., ... Ohdo, S. (2010). Glucocorticoid-dependent expression of O(6)-methylguanine-DNA methyltransferase gene modulates dacarbazine-induced hepatotoxicity in mice. *The Journal of Pharmacology and Experimental Therapeutics 333*, 782–787.

Horiguchi, M., Koyanagi, S., Hamdan, A. M., Kakimoto, K., Matsunaga, N., Yamashita, C., & Ohdo, S. (2013). Rhythmic control of the ARF-MDM2 pathway by ATF4 underlies circadian accumulation of p53 in malignant cells. *Cancer Research 73*, 2639–2649.

Horikawa, K., Yokota, S., Fuji, K., Akiyama, M., Moriya, T., Okamura, H., & Shibata, S. (2000). Nonphotic entrainment by 5-HT1A/7 receptor agonists accompanied by reduced Per1 and Per2 mRNA levels in the suprachiasmatic nuclei. *The Journal of Neuroscience 20*, 5867–5873.

Hrushesky, W. J. (1985). Circadian timing of cancer chemotherapy. *Science 228*, 73–75.

Hunter, C. A., Gabriel, K. E., Radzanowski, T., Neyer, L. E., & Remington, J. S. (1997). Type I interferons enhance production of IFN-gamma by NK cells. *Immunology Letters 59*, 1–5.

Ikeda, E., Matsunaga, N., Kakimoto, K., Hamamura, K., Hayashi, A., Koyanagi, S., & Ohdo, S. (2013). Molecular mechanism regulating 24-hour rhythm of dopamine D3 receptor expression in mouse ventral striatum. *Molecular Pharmacology 83*, 959–967.

Innominato, P. F., Focan, C., Gorlia, T., Moreau, T., Garufi, C., Waterhouse, J., ... Bjarnason, G. A. (2009). Circadian rhythm in rest and activity: A biological correlate of quality of life and a predictor of survival in patients with metastatic colorectal cancer. *Cancer Research 69*, 4700–4707.

Iwasaki, M., Koyanagi, S., Suzuki, N., Katamune, C., Matsunaga, N., Watanabe, N., ... Ohdo, S. (2015). Circadian modulation in the intestinal absorption of P-glycoprotein substrates in monkeys. *Molecular Pharmacology 88*, 29–37.

Janssen, H. L., Brouwer, J. T., van der Mast, R. C., & Schalm, S. W. (1994). Suicide associated with alfa-interferon therapy for chronic viral hepatitis. *Journal of Hepatology 21*, 241–243.

Jin, X., Shearman, L. P., Weaver, D. R., Zylka, M. J., de Vries, G. J., & Reppert, S. M. (1999). A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. *Cell 96*, 57–68.

Jones, C. R., Campbell, S. S., Zone, S. E., Cooper, F., DeSano, A., Murphy, P. J., ... Ptacek, L. J. (1999). Familial advanced sleep-phase syndrome: A short-period circadian rhythm variant in humans. *Nature Medicine 5*, 1062–1065.

Kalsbeek, A., van Heerikhuize, J. J., Wortel, J., & Buijs, R. M. (1996). A diurnal rhythm of stimulatory input to the hypothalamo-pituitary-adrenal system as revealed by timed intrahypothalamic administration of the vasopressin V1 antagonist. *The Journal of Neuroscience 16*, 5555–5565.

Kanemitsu, T., Tsurudome, Y., Kusunose, N., Oda, M., Matsunaga, N., Koyanagi, S., & Ohdo, S. (2017). Periodic variation in bile acids controls circadian changes in uric acid via regulation of xanthine oxidase by the orphan nuclear receptor PPARalpha. *The Journal of Biological Chemistry 292*, 21397–21406.

Kasukawa, T., Sugimoto, M., Hida, A., Minami, Y., Mori, M., Honma, S., ... Ueda, H. R. (2012). Human blood metabolite timetable indicates internal body time. *Proceedings of the National Academy of Sciences of the United States of America 109*, 15036–15041.

Kim, J., Matsunaga, N., Koyanagi, S., & Ohdo, S. (2009). Clock gene mutation modulates the cellular sensitivity to genotoxic stress through altering the expression of N-methylpurine DNA glycosylase gene. *Biochemical Pharmacology 78*, 1075–1082.

Koster, E. S., Rodin, A. S., Raaijmakers, J. A., & Maitland-van der Zee, A. H. (2009). Systems biology in pharmacogenomic research: The way to personalized prescribing? *Pharmacogenomics 10*, 971–981.

Koyanagi, S., Kuramoto, Y., Nakagawa, H., Aramaki, H., Ohdo, S., Soeda, S., & Shimeno, H. (2003). A molecular mechanism regulating circadian expression of vascular endothelial growth factor in tumor cells. *Cancer Research 63*, 7277–7283.

Koyanagi, S., Kusunose, N., Taniguchi, M., Akamine, T., Kanado, Y., Ozono, Y., ... Ohdo, S. (2016). Glucocorticoid regulation of ATP release from spinal astrocytes underlies diurnal exacerbation of neuropathic mechanical allodynia. *Nature Communications 7*, 13102.

Krieger, D. T. (1980). Ventromedial hypothalamic lesions abolish food-shifted circadian adrenal and temperature rhythmicity. *Endocrinology 106*, 649–654.

Krieger, D. T., Hauser, H., & Krey, L. C. (1977). Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. *Science 197*, 398–399.

Kume, K., Zylka, M. J., Sriram, S., Shearman, L. P., Weaver, D. R., Jin, X., ... Reppert, S. M. (1999). mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. *Cell 98*, 193–205.

Kusunose, N., Koyanagi, S., Hamamura, K., Matsunaga, N., Yoshida, M., Uchida, T., ... Ohdo, S. (2010). Molecular basis for the dosing time-dependency of anti-allodynic effects of gabapentin in a mouse model of neuropathic pain. *Molecular Pain 6*, 83.

Lavery, D. J., Lopez-Molina, L., Margueron, R., Fleury-Olela, F., Conquet, F., Schibler, U., & Bonfils, C. (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. *Molecular and Cellular Biology 19*, 6488–6499.

Levi, F. (2001). Circadian chronotherapy for human cancers. *The Lancet Oncology 2*, 307–315.

Levi, F., Zidani, R., & Misset, J. L. (1997). Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. International Organization for Cancer Chronotherapy. *Lancet 350*, 681–686.

Lewy, A. J. (1999). Melatonin as a marker and phase-resetter of circadian rhythms in humans. In J. Olcese (Ed.), *Melatonin after four decades. An assessment of its potentia* (pp. 425–434). New York: Kluwer Academic/Plenum Publishers.

Lohmann, C. P., Kroher, G., Bogenrieder, T., Spiegel, D., & Preuner, J. (1999). Severe loss of vision during adjuvant interferon alfa-2b treatment for malignant melanoma. *Lancet 353*, 1326.

Lundkvist, G. B., Robertson, B., Mhlanga, J. D., Rottenberg, M. E., & Kristensson, K. (1998). Expression of an oscillating interferon-gamma receptor in the suprachiasmatic nuclei. *Neuroreport 9*, 1059–1063.

Maemura, K., de la Monte, S. M., Chin, M. T., Layne, M. D., Hsieh, C. M., Yet, S. F., ... Lee, M. E. (2000). CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression. *The Journal of Biological Chemistry 275*, 36847–36851.

Matsunaga, N., Ikeda, E., Kakimoto, K., Watanabe, M., Shindo, N., Tsuruta, A., ... Ohdo, S. (2016). Inhibition of G0/G1 switch 2 ameliorates renal inflammation in chronic kidney disease. *EBioMedicine 13*, 262–273.

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*, 240–251.

Matsunaga, N., Inoue, M., Kusunose, N., Kakimoto, K., Hamamura, K., Hanada, Y., ... Ohdo, S. (2012). Time-dependent interaction between differentiated embryo chondrocyte-2 and CCAAT/enhancer-binding protein alpha underlies the circadian expression of CYP2D6 in serum-shocked HepG2 cells. *Molecular Pharmacology 81*, 739–747.

Matsunaga, N., Itcho, K., Hamamura, K., Ikeda, E., Ikeyama, H., Furuichi, Y., ... Ohdo, S. (2014). 24-hour rhythm of aquaporin-3 function in the epidermis is regulated by molecular clocks. *The Journal of Investigative Dermatology 134*, 1636–1644.

Matsunaga, N., Ogino, T., Hara, Y., Tanaka, T., Koyanagi, S., & Ohdo, S. (2018). Optimized dosing schedule based on circadian dynamics of mouse breast cancer stem cells improves the antitumor effects of aldehyde dehydrogenase inhibitor. *Cancer Research 78*, 3698–3708.

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*, 255–259.

Minami, Y., Kasukawa, T., Kakazu, Y., Iigo, M., Sugimoto, M., Ikeda, S., ... Ueda, H. R. (2009). Measurement of internal body time by blood metabolomics. *Proceedings of the National Academy of Sciences of the United States of America 106*, 9890–9895.

Moore, R. Y., & Eichler, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Research 42*, 201–206.

Murakami, Y., Higashi, Y., Matsunaga, N., Koyanagi, S., & Ohdo, S. (2008). Circadian clock-controlled intestinal expression of the multidrug-resistance gene mdr1a in mice. *Gastroenterology 135*, 1636–1644.e1633.

Nakagawa, H., Koyanagi, S., Takiguchi, T., Kuramoto, Y., Soeda, S., Shimeno, H., ... Ohdo, S. (2004). 24-hour oscillation of mouse methionine aminopeptidase2, a regulator of tumor progression, is regulated by clock gene proteins. *Cancer Research 64*, 8328–8333.

Nakagawa, H., Takiguchi, T., Nakamura, M., Furuyama, A., Koyanagi, S., Aramaki, H., ... Ohdo, S. (2006). Basis for dosing time-dependent change in the anti-tumor effect of imatinib in mice. *Biochemical Pharmacology 72*, 1237–1245.

Nishimura, K., Kato, H., & Saito, M. (1992). Effects of cyclic and continuous total enteral nutrition on 24-h rhythms of body temperature and urinary excretions. *Journal of Nutritional Science and Vitaminology (Tokyo) 38*, 117–125.

Oda, M., Koyanagi, S., Tsurudome, Y., Kanemitsu, T., Matsunaga, N., & Ohdo, S. (2014). Renal circadian clock regulates the dosing-time dependency of cisplatin-induced nephrotoxicity in mice. *Molecular Pharmacology 85*, 715–722.

Ohdo, S. (2010). Chronotherapeutic strategy: Rhythm monitoring, manipulation and disruption. *Advanced Drug Delivery Reviews 62*, 859–875.

Ohdo, S., Koyanagi, S., & Matsunaga, N. (2010). Chronopharmacological strategies: Intra- and inter-individual variability of molecular clock. *Advanced Drug Delivery Reviews 62*, 885–897.

Ohdo, S., Koyanagi, S., Suyama, H., Higuchi, S., & Aramaki, H. (2001). Changing the dosing schedule minimizes the disruptive effects of interferon on clock function. *Nature Medicine 7*, 356–360.

Ohdo, S., Makinosumi, T., Ishizaki, T., Yukawa, E., Higuchi, S., Nakano, S., & Ogawa, N. (1997). Cell cycle-dependent chronotoxicity of irinotecan hydrochloride in mice. *The Journal of Pharmacology and Experimental Therapeutics 283*, 1383–1388.

Ohdo, S., Nakano, S., & Ogawa, N. (1990). An influencing factor to predict plasma valproate concentrations: Circadian stage-dependent kinetics. *Japanese Journal of Clinical Pharmacology and Therapeutics 21*, 747–754.

Ohdo, S., Nakano, S., & Ogawa, N. (1991). Chronopharmacokinetics of valproic acid following constant-rate administration in mice. *Chronobiology International 8*, 35–43.

Ohdo, S., Ogawa, N., Nakano, S., & Higuchi, S. (1996). Influence of feeding schedule on the chronopharmacological aspects of sodium valproate in mice. *The Journal of Pharmacology and Experimental Therapeutics 278*, 74–81.

Ohdo, S., Wang, D. S., Koyanagi, S., Takane, H., Inoue, K., Aramaki, H., ... Higuchi, S. (2000). Basis for dosing time-dependent changes in the antiviral activity of interferon-alpha in mice. *The Journal of Pharmacology and Experimental Therapeutics 294*, 488–493.

Oishi, K., Koyanagi, S., Matsunaga, N., Kadota, K., Ikeda, E., Hayashida, S., ... Ohdo, S. (2010). Bezafibrate induces plasminogen activator inhibitor-1 gene expression in a CLOCK-dependent circadian manner. *Molecular Pharmacology 78*, 135–141.

Okamura, A., Koyanagi, S., Dilxiat, A., Kusunose, N., Chen, J. J., Matsunaga, N., ... Ohdo, S. (2014). Bile acid-regulated peroxisome proliferator-activated receptor-alpha (PPARalpha) activity underlies circadian expression of intestinal peptide absorption transporter PepT1/Slc15a1. *The Journal of Biological Chemistry 289*, 25296–25305.

Okazaki, F., Matsunaga, N., Okazaki, H., Azuma, H., Hamamura, K., Tsuruta, A., ... Ohdo, S. (2016). Circadian clock in a mouse colon tumor regulates intracellular iron levels to promote tumor progression. *The Journal of Biological Chemistry 291*, 7017–7028.

Okazaki, F., Matsunaga, N., Okazaki, H., Utoguchi, N., Suzuki, R., Maruyama, K., ... Ohdo, S. (2010). Circadian rhythm of transferrin receptor 1 gene expression controlled by c-Myc in colon cancer-bearing mice. *Cancer Research 70*, 6238–6246.

Okazaki, H., Matsunaga, N., Fujioka, T., Okazaki, F., Akagawa, Y., Tsurudome, Y., ... Ohdo, S. (2014). Circadian regulation of mTOR by the ubiquitin pathway in renal cell carcinoma. *Cancer Research 74*, 543–551.

Purvin, V. A. (1995). Anterior ischemic optic neuropathy secondary to interferon alfa. *Archives of Ophthalmology 113*, 1041–1044.

Reinberg, A. (1989). Chronopharmacology of corticosteroids and ACTH. In B. Lemmer (Ed.), *Chronopharmacology. Cellular and biochemical interactions* (pp. 137–178). New York: Marcel Dekker.

Reinberg, A., & Halberg, F. (1971). Circadian chronopharmacology. *Annual Review of Pharmacology 11*, 455–492.
Reinberg, A., Pauchet, F., Ruff, F., Gervais, A., Smolensky, M. H., Levi, F., ... Zidani, R. (1987). Comparison of once-daily evening versus morning sustained-release theophylline dosing for nocturnal asthma. *Chronobiology International 4*, 409–419.
Reinberg, A., Smolensky, M. H., D'Alonzo, G. E., & McGovern, J. P. (1988). Chronobiology and asthma. III. Timing corticotherapy to biological rhythms to optimize treatment goals. *The Journal of Asthma 25*, 219–248.
Ripperger, J. A., Shearman, L. P., Reppert, S. M., & Schibler, U. (2000). CLOCK, an essential pacemaker component, controls expression of the circadian transcription factor DBP. *Genes & Development 14*, 679–689.
Ritchie, A. W., Oswald, I., Micklem, H. S., Boyd, J. E., Elton, R. A., Jazwinska, E., & James, K. (1983). Circadian variation of lymphocyte subpopulations: A study with monoclonal antibodies. *British Medical Journal (Clinical Research Ed.) 286*, 1773–1775.
Saito, M., Nishimura, K., & Kato, H. (1989). Modifications of circadian cortisol rhythm by cyclic and continuous total enteral nutrition. *Journal of Nutritional Science and Vitaminology (Tokyo) 35*, 639–647.
Sakamoto, K., Nagase, T., Fukui, H., Horikawa, K., Okada, T., Tanaka, H., ... Ishida, N. (1998). Multitissue circadian expression of rat period homolog (rPer2) mRNA is governed by the mammalian circadian clock, the suprachiasmatic nucleus in the brain. *The Journal of Biological Chemistry 273*, 27039–27042.
Scott, G. M., Secher, D. S., Flowers, D., Bate, J., Cantell, K., & Tyrrell, D. A. (1981). Toxicity of interferon. *British Medical Journal (Clinical Research Ed.) 282*, 1345–1348.
Scott, G. M., Ward, R. J., Wright, D. J., Robinson, J. A., Onwubalili, J. K., & Gauci, C. L. (1983). Effects of cloned interferon alpha 2 in normal volunteers: Febrile reactions and changes in circulating corticosteroids and trace metals. *Antimicrobial Agents and Chemotherapy 23*, 589–592.
Shigeyoshi, Y., Taguchi, K., Yamamoto, S., Takekida, S., Yan, L., Tei, H., ... Okamura, H. (1997). Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. *Cell 91*, 1043–1053.
Shimba, S., Ishii, N., Ohta, Y., Ohno, T., Watabe, Y., Hayashi, M., ... Tezuka, M. (2005). Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proceedings of the National Academy of Sciences of the United States of America 102*, 12071–12076.
Shimizu, K., Sawazaki, Y., Tanaka, T., Asai, T., & Oku, N. (2008). Chronopharmacologic cancer treatment with an angiogenic vessel-targeted liposomal drug. *Biological & Pharmaceutical Bulletin 31*, 95–98.
Silas, P., Lakshmi, P. K., Ram, M., & Rao, S. (2017). Formulation technologies for chrono therapy of epilepsy: A review. *Asian Journal of Pharmacy and Pharmacology 3*, 32–40.
Silver, R., LeSauter, J., Tresco, P. A., & Lehman, M. N. (1996). A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature 382*, 810–813.
Sissung, T. M., English, B. C., Venzon, D., Figg, W. D., & Deeken, J. F. (2010). Clinical pharmacology and pharmacogenetics in a genomics era: The DMET platform. *Pharmacogenomics 11*, 89–103.
Smolensky, M. H., & Alozo, G. E. D. (1997). Progress in the chronotherapy of nocturnal asthma. In P. H. Redfern, & B. Lemmer (Eds.), *Physiology and pharmacology of biological rhythms* (pp. 205–249). Berlin Heidelberg: Springer-Verlag.
Smolensky, M. H., & Labrecque, G. (1997). Chronotherapeutics. *Pharmaceutical News 4*, 10–16.
Takiguchi, T., Tomita, M., Matsunaga, N., Nakagawa, H., Koyanagi, S., & Ohdo, S. (2007). Molecular basis for rhythmic expression of CYP3A4 in serum-shocked HepG2 cells. *Pharmacogenetics and Genomics 17*, 1047–1056.
Tanimura, N., Kusunose, N., Matsunaga, N., Koyanagi, S., & Ohdo, S. (2011). Aryl hydrocarbon receptor-mediated Cyp1a1 expression is modulated in a CLOCK-dependent circadian manner. *Toxicology 290*, 203–207.
Tei, H., Okamura, H., Shigeyoshi, Y., Fukuhara, C., Ozawa, R., Hirose, M., & Sakaki, Y. (1997). Circadian oscillation of a mammalian homologue of the Drosophila period gene. *Nature 389*, 512–516.
Terazono, H., Hamdan, A., Matsunaga, N., Hayasaka, N., Kaji, H., Egawa, T., ... Ohdo, S. (2008). Modulatory effects of 5-fluorouracil on the rhythmic expression of circadian clock genes: A possible mechanism of chemotherapy-induced circadian rhythm disturbances. *Biochemical Pharmacology 75*, 1616–1622.
Toh, K. L., Jones, C. R., He, Y., Eide, E. J., Hinz, W. A., Virshup, D. M., ... Fu, Y. H. (2001). An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science 291*, 1040–1043.
Tsurudome, Y., Koyanagi, S., Kanemitsu, T., Katamune, C., Oda, M., Kanado, Y., ... Ohdo, S. (2018). Circadian clock component PERIOD2 regulates diurnal expression of Na(+)/H(+) exchanger regulatory factor-1 and its scaffolding function. *Scientific Reports 8*, 9072.
Turek, F. W., & Allada, R. (2002). Liver has rhythm. *Hepatology 35*, 743–745.
Uematsu, T., Nakano, M., Kosuge, K., Kanamaru, M., & Nakashima, M. (1993). The pharmacokinetics of the beta 2-adrenoceptor agonist, tulobuterol, given transdermally and by inhalation. *European Journal of Clinical Pharmacology 44*, 361–364.
Ueyama, T., Krout, K. E., Nguyen, X. V., Karpitskiy, V., Kollert, A., Mettenleiter, T. C., & Loewy, A. D. (1999). Suprachiasmatic nucleus: a central autonomic clock. *Nature Neuroscience 2*, 1051–1053.
Ushijima, K., Koyanagi, S., Sato, Y., Ogata, T., Matsunaga, N., Fujimura, A., & Ohdo, S. (2012). Role of activating transcription factor-4 in 24-hour rhythm of serotonin transporter expression in the mouse midbrain. *Molecular Pharmacology 82*, 264–270.
Wada, E., Koyanagi, S., Kusunose, N., Akamine, T., Masui, H., Hashimoto, H., ... Ohdo, S. (2015). Modulation of peroxisome proliferator-activated receptor-alpha activity by bile acids causes circadian changes in the intestinal expression of Octn1/Slc22a4 in mice. *Molecular Pharmacology 87*, 314–322.
Wirz-Justice, A., Cajochen, C., & Nussbaum, P. (1997). A schizophrenic patient with an arrhythmic circadian rest-activity cycle. *Psychiatry Research 73*, 83–90.
Wirz-Justice, A., Werth, E., Savaskan, E., Knoblauch, V., Gasio, P. F., & Muller-Spahn, F. (2000). Haloperidol disrupts, clozapine reinstates the circadian rest-activity cycle in a patient with early-onset Alzheimer disease. *Alzheimer Disease and Associated Disorders 14*, 212–215.
Wollnik, F., Brysch, W., Uhlmann, E., Gillardon, F., Bravo, R., Zimmermann, M., ... Herdegen, T. (1995). Block of c-Fos and JunB expression by antisense oligonucleotides inhibits light-induced phase shifts of the mammalian circadian clock. *The European Journal of Neuroscience 7*, 388–393.
Yamaguchi, S., Mitsui, S., Yan, L., Yagita, K., Miyake, S., & Okamura, H. (2000). Role of DBP in the circadian oscillatory mechanism. *Molecular and Cellular Biology 20*, 4773–4781.
Yan, L., Miyake, S., & Okamura, H. (2000). Distribution and circadian expression of dbp in SCN and extra-SCN areas in the mouse brain. *Journal of Neuroscience Research 59*, 291–295.
Youan, B. B. (2004). Chronopharmaceutics: Gimmick or clinically relevant approach to drug delivery? *Journal of Controlled Release 98*, 337–353.
Youan, B. B. (2010). Chronopharmaceutical drug delivery systems: Hurdles, hype or hope? *Adv Drug Deliv Rev 62*, 898–903.
Zhou, S. F., Di, Y. M., Chan, E., Du, Y. M., Chow, V. D., Xue, C. C., ... Duan, W. (2008). Clinical pharmacogenetics and potential application in personalized medicine. *Current Drug Metabolism 9*, 738–784.
Zylka, M. J., Shearman, L. P., Weaver, D. R., & Reppert, S. M. (1998). Three period homologs in mammals: Differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. *Neuron 20*, 1103–1110.