Review

Chronopharmaceutics: Pharmaceutics Focused on Biological Rhythm

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The circadian pacemaker of mammals resides in the paired suprachiasmatic nuclei (SCN) and influences a multitude of biological processes, including the sleep-wake rhythm. Clock genes are the genes that control the circadian rhythms in physiology and behavior. Twenty-four hour rhythm has been demonstrated for the function of physiology and the pathophysiology of diseases. The effectiveness and toxicity of many drugs vary depending on dosing time. Such chronopharmacological phenomena are influenced by not only the pharmacodynamics but also the pharmacokinetics of medications. Thus, knowledge of the 24 h rhythm in the risk of disease plus evidence of 24 h rhythm dependencies of drug pharmacokinetics, effects, and safety constitutes the rationale for pharmacotherapy. One approach to increasing the efficiency of pharmacotherapy is the administration of drugs at times at which they are most effective and/or best tolerated. Drugs for several diseases are still given without regard to the time of day. Identification of a rhythmic marker for selecting dosing time will lead to improved progress and diffusion of chronopharmacotherapy. The mechanisms underlying chronopharmacological findings should be clarified from the viewpoint of clock genes. On the other hand, several drugs have an effect on the circadian clock. The knowledge of interactions between the circadian clock and a drug should be very useful in clinical practice. Therefore, the aim of this review is to provide an overview of the dosing time-dependent alterations in therapeutic outcome and safety of drugs. The mechanisms are introduced from the viewpoint of pharmaceutics.

Key words chronopharmaceutics; chronopharmacology; chronotherapy; circadian rhythm; biological clock; clock gene

INTRODUCTION

The mammalian circadian pacemaker resides in the suprachiasmatic nuclei (SCN) and influences a multitude of biological processes, including the sleep-wake rhythm.[1] The circadian clock acts like a multifunction timer to regulate homeostatic systems such as sleep and activity, hormone levels, appetite, and other bodily functions with 24 h cycles. Clock genes are identified as the genes that ultimately control a vast array of circadian rhythms in physiology and behavior.[2]

The individualization of pharmacotherapy has been performed mainly by monitoring drug concentrations. However, a pharmacogenetic approach such as genetic diagnosis has become a very attractive field due to the rapid progress of molecular biology.[3] Consequently, dosage adjustment has been based on the interindividual differences in drug pharmacokinetics. However, intraindividual variability as well as interindividual variability should be considered in order to achieve further improvement of rational pharmacotherapy. This is because many drugs vary in potency and/or toxicity associated with the rhythmicity of biochemical, physiological and behavioral processes.[4—8] Theoretically, it has been argued that drug administration at certain times of the day should improve the outcome of pharmacotherapy.

Biological rhythms not only impact the function of physiology, but also the pathophysiology of diseases.[4—8] Chronopharmacology is the investigative science that elucidates the biological rhythm dependencies of medications. The effectiveness and toxicity of many drugs vary depending on dosing time associated with 24 h rhythms of biochemical, physiological and behavioral processes under the control of circadian clock. Such chronopharmacological phenomena are influenced by not only the pharmacodynamics but also pharmacokinetics of medications. The knowledge of 24 h rhythm in the risk of disease plus evidence of 24 h rhythm dependencies of drug pharmacokinetics, effects, and safety constitutes the rationale for pharmacotherapy (chronotherapy). Chronotherapy is especially relevant in the following cases.[4—8] The risk and/or intensity of the symptoms of disease vary predictably over time as exemplified by allergic rhinitis, arthritis, asthma, myocardial infarction, congestive heart failure, stroke, and peptic ulcer disease. From the viewpoint of pharmaceutics, the application of biological rhythm to pharmacotherapy may be accomplished by the appropriate timing of conventionally formulated tablets and capsules, and special drug delivery systems to synchronize drug concentrations to rhythms in disease activity.[7] Pharmaceutical companies have focused on the investigation of underlying mechanisms as well as the conduct of multicenter clinical studies involving numerous patients with the purpose of devising chronotherapeutic interventions with a variety of medications. New technology for delivering medications precisely in a time-modulated fashion by bedside or ambulatory pumps is being developed to manage human diseases. Chronopharmacologists have the responsibility to carefully evaluate and use this new technology to ensure the devices and clinical findings are well accepted by colleagues currently involved in more classical research.

Drugs for several diseases are still given without regard to the time of day, because chronopharmacological findings have not been systematically summarized in an applicable format for clinical practice and a reference rhythm for circadian timing of medications has not been clarified.[8] Identification of a rhythmic marker for selecting dosing time will lead to improved progress and diffusion of chronopharmacotherapy. The mechanisms underlying chronopharmacological findings should be clarified from the viewpoint of clock genes. On the other hand, several drugs have an effect on the circadian clock. The knowledge of interactions between the

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circadian clock and drug should be very useful for clinicians.

The aim of this review is to provide an overview of the dosing time-dependent alterations in therapeutic outcome and drug safety. The underlying mechanisms and usefulness are introduced from the viewpoint of pharmaceutics. Furthermore, the regulatory system of biological rhythm and the possibility of pharmacotherapy are introduced from the viewpoint of clock genes.

BIOLOGICAL CLOCK

The SCN of the anterior hypothalamus is the site of the circadian pacemaker in mammals.[1)] Like any timing system, the circadian clock is made up of three components[1,6)]: an input pathway adjusting the time, a central oscillator generating the circadian signal, and an output pathway manifesting itself in circadian physiology and behavior. The daily changes in light intensities are thought to be 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. A great deal of research has shown that the inherited period of the human pacemaker clock is not precisely 24 h. In fact, in most people, it is somewhat longer, being closer 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. Clock genes were identified as the genes that ultimately control a vast array of circadian rhythms in physiology and behavior.[2)] The clock genes are expressed not only in the SCN, but also in other brain regions and various peripheral tissues.

24 H RHYTHMS IN PHYSIOLOGICAL FUNCTIONS AND DISEASES

Chronobiological approach has clarified the presence of 24 h rhythms in physiological functions and diseases. The examples described below show the approximate peak time of 24 h rhythms relative to the diurnally active human.[4—8)] The rhythms of serum cortisol, aldosterone, testosterone, platelet adhesiveness, blood viscosity, and NK-cell activity show a peak during the initial hours of daytime. Hematocrit is the greatest and airway caliber (FEV1) is the best around the middle and afternoon hours, respectively. The peaks in insulin, cholesterol, triglycerides, platelet numbers, and uric acid occur later during the day and evening. The rhythms of basal gastric acid secretion, white blood cells (WBC), lymphocytes, prolactin, melatonin, eosinophils, adrenal corticotrophic hormone (ACTH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) shows peaks at specific times during the nighttime.

Twenty-four hour rhythms in the processes that make up the pathophysiology of diseases cause prominent day–night patterns in the manifestation and severity of many medical conditions as shown in Fig. 1.[4—8)] The sneezing, runny nose, and stuffy nose in allergic and infectious rhinitis are worst in the morning upon getting out of bed. The onset of migraine headaches is most frequent in the morning around the time of awakening. The symptoms of rheumatoid arthritis are the worst first thing in the morning, while those of osteoarthritis are the worst later in the day. The morbidity and mortality associated with myocardial infarction are greatest during the initial hours of daytime. The incidences of thrombotic and hemorrhagic stroke are the greatest in the morning around the time diurnal activity is commenced. The ischemic events, chest pain, and ST-segment depression of angina are the strongest during the initial three to five hours of daytime. The pain and gastric distress experienced at the onset of peptic ulcer disease and its acute exacerbation are most prevalent 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 nocturnally. The manifestation of ST-segment elevation in Prinzmetal's angina is most frequent during the middle to later half of the night. The risk of an asthma attack is greatest during nighttime.

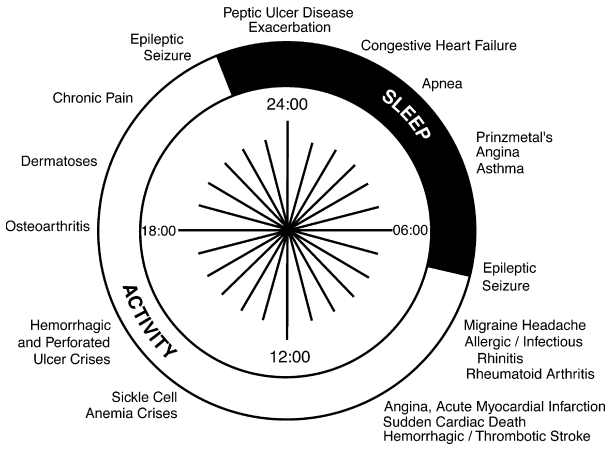

Fig. 1. 24 h Clock Diagram of the Approximate Time, in Humans Following the Diurnal Activity/Nocturnal Sleep Routine, when Symptoms or Events of Diseases Are Worst or Most Frequent[4—8)]

CHRONOTHERAPY

The knowledge of 24 h rhythm in the risk of disease plus evidence of 24 h rhythm dependencies of drug pharmacokinetics, pharmacodynamics, effects, and safety constitutes the rationale for pharmacotherapy (chronotherapy).[4—8)] One approach for increasing the efficiency of pharmacotherapy is the administration of drugs at times at which they are most effective and/or best tolerated. The chronotherapy of a medication may be accomplished by the appropriate timing of conventionally formulated tablets and capsules, and special drug delivery systems to synchronize drug concentrations to rhythms in disease activity. Chronotherapy is especially relevant in the following cases. The risk and/or intensity of the symptoms of disease vary predictably over time as exemplified by allergic rhinitis, arthritis, asthma, myocardial infarction, congestive heart failure, stroke, and peptic ulcer disease. The pharmacokinetics and pharmacodynamics of a medication vary depending on biological rhythms. The therapeutic-to-toxicity ratio of a medication varies predictably according to chronobiological determinants as exemplified by antitumor medications. The goal of pharmacotherapy is hormonal substitution to mimic the rhythmic variation of hormone levels in healthy individuals. Also on the horizon are

drugs to fix broken biological clocks, which is perhaps a factor in all illnesses, in the opinion of some physicians. Several examples for chronopharmacotherapy are described below[4—12]: The morning daily or alternate-day dosing strategy for methylprednisolone constitutes the first chronotherapy to be incorporated into clinical practice: Evening, once-daily dosing of specially formulated theophylline tablets for the treatment of nocturnal asthma: 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 hypertension: Evening administration of hydroxymethylglutaryl (HMG)-CoA-reductase antagonists for the management of hyperlipidemia: Evening, once-daily dosing of conventional H_2-receptor antagonists or morning once-daily administration of proton-pump antagonist tablet medications for the management of peptic ulcer disease: Before-bedtime administration of hypnotics for sleep induction and maintenance: Morning application of testosterone drug-delivery patch systems to achieve a physiologic androgen-replacement therapy: Programmed-in-time infusion of antitumor medications according to biological rhythms to moderate toxicity and enhance dose-intensity in cancer treatment: Programmed-in-time administration of tocolytic medication relative to the 24 h rhythm in uterine contractility to avert preterm labor and birth.

INFLUENCE OF BIOLOGICAL RHYTHMS ON PHARMACODYNAMICS AND PHARMACOKINETICS

Biological rhythms not only impact the pathophysiology of diseases, but also the pharmacokinetics and pharmacodynamics of medications. Chronopharmacology is the investigative science that elucidates the biological rhythm dependencies of medications.

Chronopharmacodynamics Biological rhythms at the cellular and subcellular levels can give rise to significant dosing-time differences in the pharmacodynamics of medications that are unrelated to their pharmacokinetics.[4—8,13—16] This phenomenon is termed chronesthesy. Rhythms in receptor number or conformation, second messengers, metabolic pathways, and/or free-to-bound fraction of medications help explain this phenomenon. For example, the antitumor effect of interferon-β (IFN-β) and the antiviral effect and lymphocyte stimulating effect of IFN-α in mice are more efficient during the early rest phase than during the early active phase.[13,14] The dosing schedule-dependent effect of IFN-β or IFN-α is also closely related to that of IFNs receptors and ISGF expression in tumor cells or lymphocytes.

Imatinib mesylate is a molecule that inhibits the function of various receptors with tyrosine kinase activity, such as Abl, the bcr-abl chimeric product, KIT, and platelet-derived growth factor (PDGF) receptors.[15] The influence of dosing time on the ability of imatinib to inhibit tumor growth in mice has been investigated. The growth of tumor cells implanted in mice is more severely inhibited by the administration of imatinib during the early rest phase than during the early active phase. The dosing time-dependency of antitumor effects is parallel to that of the imatinib-induced antiangiogenic effect. The inhibitory effect of imatinib on tyrosine kinase activity of PDGF receptors, but not of KIT and Abl, varies according to its administration time. The anti-tumor efficacy of imatinib is enhanced by administering the drug when PDGF receptor activity is increased. The potent therapeutic efficacy of the drug could be expected by optimizing the dosing schedule.

Chronopharmacokinetics Many physiological factors such as gastrointestinal, cardiovascular, hepatic, and renal changes vary according to time of day as shown in Table 1.[7,8,17,18] Such changes contribute to circadian stage-dependent changes in the fate of drugs in the organism (*e.g.*, absorption, distribution, metabolism, and elimination). Chronopharmacokinetic studies have been reported 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 pharmacokinetics of a drug.

Drug Absorption: Circadian changes in drug absorption have been demonstrated for several orally administered drugs in humans. Gastric acid secretion and pH, motility, gastric emptying time, and gastrointestinal blood flow vary according to the time of day.[19,20] Such changes may contribute to the dosing time-dependent difference of drug absorption. For example, circadian changes in pH may induce circadian modifications of drug ionization according to its physicochemical properties. To clarify the role of gastric emptying in drug absorption, gastric emptying rates were compared between morning (08:00) and evening (20:00) in 16 healthy male subjects.[21] Gastric emptying half-times for the evening meal were significantly longer for solids but not liquids compared with those of the morning meal. The increase in evening meal emptying time may account for the evening delay in reaching peak plasma concentrations documented for several drugs.

The dosing time-dependent difference of drug absorption is influenced by the physicochemical properties of a drug (lipophilicity or hydrophilicity).[22] The circadian changes in drug absorption are significant for lipophilic drugs, while such changes have not been demonstrated for hydrophilic drugs.[23] Such variations may be related to the physicochemical properties of a drug, since most lipophilic drugs seem to be absorbed faster in the morning as compared to evening. To the contrary, the absorption processes of highly water-soluble drugs do not change depending on dosing time. The

Table 1. Possible Physiological Factors Influencing Circadian Stage-Dependent Pharmacokinetics of Drugs (*e.g.*, Absorption, Distribution, Metabolism, and Elimination)[7,8,17,18]

Absorption:
(Oral)
Gastric pH, gastric motility, gastric emptying time, gastrointestinal blood flow, transporter
(Parenteral)
Transdermal permeability, ocular permeability, pulmonary permeability
Distribution:
Blood flow, albumin, α1-acid glycoprotein, red blood cells, transporter
Metabolism:
Liver enzyme activity, hepatic blood flow, gastrointestinal enzyme
Elimination:
(Renal, biliary, intestinal)
Glomerular filtration, renal blood flow, urinary pH, electrolytes, tubular resorption, transporter

Each process is also influenced by life style such as active-rest cycle, posture and eating schedule, and physicochemical properties of a drug (lipophilicity or hydrophilicity).

mechanisms underlying the chronopharmacokinetics of lipophilic drugs involve a faster gastric emptying time and a higher gastrointestinal perfusion in the morning.[20)]

Feeding conditions contribute to dosing time-dependent differences in drug absorption. For example, the effect of meal condition on circadian changes in valproic acid (VPA) kinetics was investigated in 16 healthy men.[24)] VPA tablets (800 mg) were given orally on two occasions in the morning (08:30) or in the evening (20:30). First, eight subjects had a light breakfast in the morning and a heavy dinner in the evening in accordance with usual amount of food consumed by the subject. The mean peak plasma concentration (C_{max}) was significantly higher, the time to peak concentration (t_{max}) was shorter, and the absorption rate (K_a) was larger after the morning dose than after the evening dose. Second, the size and content of the breakfast and dinner meals were prepared in the same manner as the standard breakfast for the subjects. In other words, 8 subjects ate the same light meal in the morning and in the evening, respectively. No significant circadian change was demonstrated for VPA kinetics under this same meal conditions. Thus, differences in the meal between morning and evening in our daily life play a major role in the mechanism underlying the circadian changes of VPA absorption. Certainly, feeding conditions are circadian time-dependent in usual resting/activity conditions, but they must be considered to avoid a masking.

Drug absorption by route of administration other than oral is also influenced by biological rhythms.[17,18)] For example, skin permeability shows circadian time-dependent differences in drug absorption. The temporal variation in drug penetration has previously been demonstrated for local anesthetic agents.[25)] Such phenomena should be considered in specific drug penetration through the skin using patches, since transdermal devices are applied 24 h a day. Ocular absorption of topically applied beta-blockers has also been demonstrated to have circadian time-dependent differences in pigmented rabbits.[26)] Thus, all factors including route of administration, feeding conditions, posture and galenic formulation should be considered, when taking into account the biological rhythms.

Drug Distribution: Circadian changes in biological fluids and tissues related to drug distribution are documented to vary according to time of day.[23)] Blood flow depends on several regulatory factors, including the sympathetic and parasympathetic systems whose activities are known to be circadian time-dependent with a predominant diurnal effect of the sympathetic system.[27)] Thus, diurnal increases and nocturnal decreases in blood flow and local tissue blood flows may explain a possible difference in drug distribution depending on dosing time. Plasma proteins such as albumin or alpha 1 glycoprotein acid have been documented to be circadian time-dependent.[28,29)] The plasma concentrations of albumin and alpha 1 glycoprotein acid show peaks around noon. As a result, daily variations have been reported for drug protein binding.[7,8)] Clinically significant consequences of such temporal changes in drug binding are relevant only for drugs which are highly bound. Thus, temporal variations in plasma drug binding may have clinical implications only for drugs characterized by a high degree of protein binding and a small volume of distribution. For example, the effect of dosing time on diazepam kinetics was investigated in 28 healthy men.[30)] A 5-mg dose of diazepam was given orally or intravenously in the morning (09:30) or in the evening (20:30) under postprandial conditions or after a 9 h fast. After oral doses under postprandial conditions, the mean peak total diazepam concentration in plasma was higher and the time to peak concentration was faster after morning dosing than after evening dosing. Intravenous diazepam does not eliminate the time-dependent changes in diazepam kinetics occurring soon after injection under postprandial conditions, although it diminishes the difference. During the 9 h fast, the time-dependent changes in diazepam kinetics were marked, especially after intravenous injection. The diazepam free fraction was lower 0.5 h after intravenous dosing in the morning. A negative correlation was demonstrated between the diazepam free fraction and total diazepam plasma concentration. Thus, diurnal variations in the rate of drug distribution because of alterations in protein binding as well as in absorption from the gastrointestinal tract contribute to the time-dependent changes in diazepam kinetics.

As a particular concern in drug binding to red blood cells, circadian time-dependent changes in the passage of drug into red blood cells have been demonstrated for drugs such as local anesthetics, indomethacine, and theophylline.[31)] Furthermore, P-glycoprotein (Pgp), the product of the multidrug resistance (mdr) gene which contributes to renal, biliary, and intestinal elimination of drugs, and the intestinal H(+)/peptide cotransporter 1 (PEPT1) play important roles as a nutrient and drug transporter function as a xenobiotic transporter and exhibit 24 h variation.[32,33)]

Drug Metabolism: Hepatic drug metabolism seems to depend on liver enzyme activity and/or hepatic blood flow.[4—8)] Both factors show circadian time-dependent differences. Enzyme activities show circadian time-dependent differences in many tissues such as brain, kidney, and liver.[34,35)] However, these data were obtained in animals. Such circadian changes in enzyme activity have not been reported in humans. Several chronopharmacological studies have indirectly investigated temporal variations in hepatic drug metabolism by evaluating the chronopharmacokinetics of drugs and their metabolites. Thus, conjugation, hydrolysis and oxidation show circadian time-dependent differences.[4—8)] For example, circadian variations in the urinary 6 beta-hydrocortisol to cortisol ratio in humans show those in cytochrome CYP3A activity.[36)] As for metabolic phenotype, the effect of diurnal variation has been demonstrated for debrisoquine metabolic phenotyping, with the slowest rate of metabolism occurring during the daytime.[37)] For drugs with a high extraction ratio, hepatic metabolism depends on hepatic blood flow. Circadian variations in hepatic blood flow induce changes in liver perfusion and, thus, temporal variations in the clearance of such drugs. As an example, daily variations in hepatic blood flow investigated by indocyanine green (ICG) clearance in healthy volunteers show the highest values in the early morning.[38)] Intravenous injections of ICG were given to 10 healthy subjects at 02:00, 08:00, 14:00 and 20:00. ICG plasma half-life, plasma clearance and estimated hepatic blood flow (EHBF) varied significantly with time of day with EHBF being the greatest at 08:00. The circadian rhythm in EHBF should be considered when evaluating the pharmacokinetics of high-clearance drugs at different times of the day. Such temporal changes are of particular interest when attempting to explain the

mechanisms for the dosing time-dependent pharmacokinetics of drugs with a high hepatic extraction ratio.

Drug Elimination: Renal physiological functions such as glomerular filtration, renal blood flow, urinary pH and tubular resorption show circadian time-dependent differences with higher values during daytime.[39] These rhythmic variations in renal functions may contribute to circadian dependent changes in drug urinary excretion. The rhythmicity in urinary pH modifies drug ionization and may explain why acidic drugs are excreted faster after evening administration, as demonstrated for sodium salicylate[40] and sulfasymazine.[41] Such variations are obviously more pronounced for hydrophilic drugs. For example, sodium salicylate (1 g solution/24 h) was administered to six healthy volunteers at one of four different circadian times, such as 07:00, 11:00, 19:00 and 23:00.[40] Urine was collected at 4 h intervals for 48 h following drug ingestion. By measuring the height of the peak excretion and the span necessary to reach the peak, it was found that salicylate was excreted faster into the urine, reached higher values sooner, and fell off faster when the drug was ingested between 19:00 and 23:00 as compared to at other times. The mechanisms seem to be related to variations in drug ionization according to feeding conditions. The influence of food on the chronopharmacokinetics of antibiotics shows that feeding restriction may modify the chronopharmacokinetics of antimicrobial agents by modifying urinary pH.[42,43]

Therapeutic Drug Monitoring (TDM) The circadian stage-dependent changes in kinetic aspects of drugs have been reported for many drugs as a function of the time of day of administration. The intraindividual variability, such as circadian rhythm, as well as interindividual variability becomes influencing factors in dosage adjustment. For example, the influence of the dosing time on the accuracy in predicting plasma VPA concentrations at steady state was investigated.[44] VPA at a dose of 400 mg for 9 d on a twice-daily basis (08:30 and 20:30) was administered to eight healthy male volunteers. The circadian changes in VPA kinetics occur at the absorption phase. The prediction of VPA concentrations at 2 h (around Cmax) and at 12 h after both the morning and the evening dose on the ninth day was performed using the individual subject's pharmacokinetic parameters and population pharmacokinetic parameters (Bayesian method) obtained from the morning trial on the eighth day. The predictive accuracy for VPA concentrations around Cmax after the morning dose was better. But the predictive accuracy for VPA concentrations after the evening dose was significantly biased toward overestimation. The individual pharmacokinetic parameters obtained from the morning trial became better sources for the prediction of VPA concentrations around Cmax after the morning dose, but worse sources after the evening dose. The predictive performance based on the Bayesian approach also shows a finding similar to that based on individual pharmacokinetic parameters. The overestimation of the target points around Cmax after the evening dose was closely related to that of the Ka value produced by using population parameters obtained from the morning trial. Therefore, the time in the circadian stage at which VPA is administered is important for exact evaluation of the predictive accuracy. The importance of circadian stage-dependent pharmacokinetics has been demonstrated for the accuracy in predicting a single serum theophylline concentration using the Bayesian method.[45] The predictive accuracy of drug concentration is improved when the time-of-day matched pharmacokinetic parameters are used. The intraindividual variability, such as circadian rhythm, as well as interindividual variability should be considered in dosage adjustment.

Chrono-Drug Delivery System (DDS) The effectiveness and toxicity of many drugs vary depending on the relationship between the dosing schedule and the 24 h rhythms of biochemical, physiological and behavioral processes. In addition, several drugs can cause alterations to the 24 h rhythms leading to illness and altered homeostatic regulation. The alteration of biological rhythm is a new concept of adverse effects. The disruption of circadian rhythm can be minimized by optimizing the dosing schedule.[46] Many reports demonstrate the rationale behind chronotherapy.[47—49] However, much of the drug delivery research over the past decades has focused on a constant drug release rate. The reason why the majority of DDS is designed with little emphasis on proven oscillatory phenomena may be found in drug delivery limitations. Chronopharmaceutics should address these new challenges in DDS. Due to advances in chronobiology, chronopharmacology, and global market constraints, the traditional goal of pharmaceutics such as a constant drug release rate is becoming obsolete. However, the major bottleneck in the development of DDS that match the circadian rhythm (Chrono-DDS) may be the availability of appropriate technology. The last decade has witnessed the emergence of Chrono-DDS for several diseases. The increasing research interest surrounding Chrono-DDS may lead to the creation of a new sub-discipline in pharmaceutics known as chronopharmaceutics.

As shown in Table 2, the technologies in chronopharmaceutics includes: CONTIN®, physico-chemical modification 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.[8,47,50] A major aim of chronopharmaceutics is to deliver the drug in higher concentrations during the time of greatest need and in lesser concentrations when the need is less to minimize side effects.

As examples of Chrono-DDS on the market, there are compounds such as 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 FDA electronic orange book,[50] specific product package inserts, and United States patent and specific pharmaceutical company websites. Future developments in chronopharmaceutics may be made at the interface of other emerging disciplines such as system biology and nanomedicine. Such novel and more biological approaches to drug delivery may lead to safer and more efficient disease therapy in the future.

MOLECULAR CLOCK MECHANISM UNDERLYING DISEASE, PHARMACODYANAMICS AND PHARMACOKINETICS

Clock genes are the genes that control the circadian

Table 2. Examples of Chrono-Drug Delivery Systems on the Market in U.S.A. and Japan[8,47,50]

Active pharmaceutical ingredient (API)	Proprietary name dosages form	Proprietary chronopharmaceutical technology	Disease
U.S.A.			
Theophylline	Uniphyl® extended release tablets	CONTIN®	Asthma
Famotidine	Pepcid® tablets	Physico-chemical modification of API	Ulcer
Simvastatin	Zocor® tablets	Physico-chemical modification of API	Hyperlipidemia
Verapamil HCL	Covera-HS® extended release tablets	OROS®	Hypertension
Verapamil HCL	Verelan® PM extended release capsules	CODAS®	Hypertension
Diltiazem HCL	Cardizem® LA	CEFORM®	Hypertension
Verapamil HCL	extended release tablets		
Propranolol HCL	InnoPran® XL	DIFFUCAPS®	Hypertension
Verapamil HCL	extended release capsules		
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 increased bronchoconstriction in the early morning. Famotidine is used for ulcers associated with increased gastric acid secretion in the evening. Simvastatin is used for hypercholesterolemia associated with increased cholesterol synthesis in the evening. Verapamil, Diltiazem and Propranolol are used for hypertension associated with increased blood pressure in the morning. Tulobuterol is used for asthma associated with increased bronchoconstriction in the early morning.

rhythms in physiology and behavior.[2] Three mammalian clock genes (*Per1*, *Per2* and *Per3*) are rhythmically expressed in the SCN. *Per1* and *Per2* are induced in response to light.[51] In particular, *Per1* induction is considered to be an initial event in light-induced resetting and entrainment of the circadian biological clock.[52] The transcriptional machinery of the core clockwork regulates a clock-controlled output rhythm as shown in Fig. 2.[53] Namely, CLOCK-BMAL1 heterodimers act through an E box enhancer to activate the transcription of *Pers*, *vasopressin* and *Dbp* mRNA showing a specific output function from the SCN to periphery.[53—55] This activation can be inhibited by the PER and CRY proteins.[56] A circadian rhythm of *Pers* mRNA expression is discovered not only in the SCN but also in other tissues.[57] The circadian rhythm in the periphery is governed by that in the SCN, since the circadian rhythm in physiological function and *Pers* mRNA expression are abolished in SCN-lesioned rats[57] and *Clock* mutant mice.[53] 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 poorly understood but are likely to involve both nervous and humoral signals.[58—60] The regulatory system of biological rhythm should be clarified in detail from the viewpoint of clock genes. Knowledge concerning clock genes should be applied to clinical practice. Therefore, I will discuss the regulatory system of biological rhythm from the viewpoints of clock genes and the possibility of pharmacotherapy based on clock genes.

Sleep disorders in humans are associated with a genetic mutation affecting circadian clock function. Familial advanced sleep-phase syndrome (FASPS) has been documented.[61] Affected individuals experience early evening sleepness and early morning awakening. Individuals with FASPS have a circadian period about an hour shorter than normal. In one of the FASPS families, the mutant gene is *hPer2*, the human homolog of *mPer2*.[62] The *hPer2* mutation changes serine 662 to a glycine (S662G). This occurs in a region of hPER2 homologous to the casein kinase I epsilon (CKIε) binding region of mPER1 and mPER2. Serine 662 is

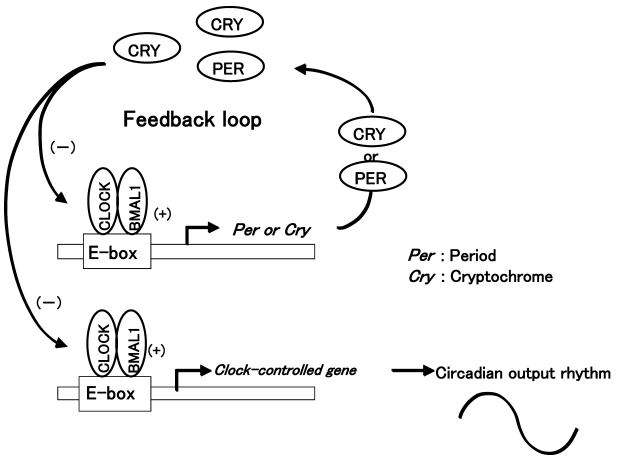

Fig. 2. Simplified Model for a Feedback Loop Regulation of Clock Gene and the Relative Clock-Controlled Genes[2,53]

Heterodimers of CLOCK and BMAL1 activate transcription of clock genes and clock-controlled genes. The CRY proteins shut down CLOCK-BMAL1 transcription 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, which include PER3, DBP (D-element binding protein), and AVP (arginine vasopressin), transduce the core oscillation to downstream output systems.

in fact part of a 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 variant in human sleep behavior can be attributed to a missense mutation in a clock component, hPER2, which alters the circadian period.

The onset of myocardial infarction occurs frequently in the early morning, and it may partly result from the circadian rhythm of fibrinolytic activity. Plasminogen activator inhibitor-1 (PAI-1) activity shows a circadian rhythm.[63] Basic helix-loop-helix (bHLH)/PAS domain transcription factors play a crucial role in controlling the biological clock that controls circadian rhythm. A novel bHLH/PAS protein, cycle-like factor (CLIF), is isolated from human umbilical vein endothelial cells. CLIF shares high homology with

Drosophila CYCLE, one of the essential transcriptional regulators of circadian rhythm. In endothelial cells, CLIF forms a heterodimer with CLOCK and up-regulates the *PAI-1* gene through E-box sites. Furthermore, *Per2* and *Cry1* inhibit the *PAI-1* promoter activation by the CLOCK:CLIF heterodimer. In other words, CLIF regulates the circadian rhythm of *PAI-1* gene in endothelial cells. In addition, the results potentially provide a molecular basis for the morning onset of myocardial infarction. Although BMAL1 is a transcription factor controlling circadian rhythm, it plays important roles in the regulation of adipose differentiation and lipogenesis in mature adipocytes.[64]

Angiogenesis is essential for tumor growth and metastasis. The inhibition of angiogenesis has emerged as a therapy to treat various cancers. Methionine aminopeptidase2 (MetAP2) plays an important role in the growth of endothelial cells during the tumor angiogenesis stage. MetAPs show a circadian rhythm in implanted tumor masses in mice.[65] The transcription of the *MetAP2* promoter is enhanced by the CLOCK:BMAL1 heterodimer, and its activation is inhibited by PER2 or CRY1. In sarcoma 180-bearing mice, the pattern of binding of CLOCK and BMAL1 to the E-box and transcription of the *MetAP2* promoter shows a circadian rhythm with higher levels from the mid-rest to early active phase. The pattern of *MetAP2* transcription is closely associated with that of *MetAP2* mRNA expression in three types of tumor-bearing mice. MetAP2 protein expression varies with higher levels from the late-active to early rest phase. That is to say, 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 MetAP2 activity increases. A similar finding has been demonstrated for the hypoxia-induced expression of vascular endothelial growth factor (VEGF) playing a key role in tumor-induced angiogenesis.[66] Thus, the regulatory mechanisms underlying 24 h rhythm of pharmacodynamics should be also clarified from the viewpoint of clock genes.

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 circadian clock control. Triple mutant mice are born at expected Mendelian ratios, but are epilepsy prone, age at an accelerated rate, and die prematurely.[67] The PAR bZip transcription factors DBP, TEF, and HLF accumulate in a highly circadian manner in several peripheral tissues, including liver and kidney. To identify PAR bZip target genes whose altered expression might contribute to the high morbidity and mortality of PAR bZip triple knockout mice, the liver and kidney transcriptomes of these animals have been compared with those of wild-type or heterozygous mutant mice. The disruption of these three genes in mice alters the gene expression patterns of many proteins involved in drug metabolism and in liver and kidney responses to xenobiotic agents. For example, molecular clock work contributes to produce the rhythmicity in xenobiotic metabolic enzymes such as CYP3A4 and CYP2E1, and a xenobiotic transporter such as MDR.[68—70] Thus, the regulatory mechanisms underlying the 24 h rhythm of pharmacokinetics should be also clarified from the viewpoint of clock genes.

Several drugs cause alterations in the 24 h rhythms of biochemical, physiological and behavioral processes.[46,71,72] The alteration of rhythmicity is sometimes associated with therapeutic effects, or may lead to illness and altered homeostatic regulation. Interferons (IFNs) have been widely used as antiviral and antitumor agents. However, IFNs cause adverse neuropsychiatric effects such as depression and neurosis and they are reported to sometimes lead to suicide.[73—75] The mechanism has been clarified from the viewpoint of the disruptive effect of the drug on the clock genes in mice. The rhythmicity of locomotor activity, body temperature and clock genes are severely blunted by the repetitive administration of interferon-α (IFN-α). IFN-α influenced both the SCN and periphery. Interestingly, an inhibitory effect of mRNA expression of each clock gene in the SCN is observed by the repetitive administration of IFN-α during the early active phase, but not the early rest phase. The observations for humans described above correspond well to the findings indicating that alteration of the clock genes is induced by IFN-α administration during the early active phase in nocturnally active rodents. Furthermore, the 24 h dependency of the disruptive effect of IFN-α on clock genes in SCN may be applicable to other drugs as shown in the case of 5-FU.[76] Thus, alteration of the clock function, a new concept of adverse effects, can be overcome by devising a dosing regimen that minimizes adverse drug effects on clock function.

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 conditions and several drugs.[52,61,71,77] SCN neurons receive information about the light intensity in the environment *via* direct synaptic connections with the retina, which adapts the phase of the SCN oscillator to the photoperiod.[78] 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.[52] The responsiveness of *Per1* mRNA to light is closely related to behavioral phase delays induced by light. Also, the acute and circadian time-dependent reduction of *Per1* and/or *Per2* mRNA in the hamster SCN by 5-HT1A/7 receptor agonists is strongly correlated with the phase resetting in response to the drug.[79] A variety of physiological rhythmic variables are influenced by the cyclic variation of environmental factors.[80] One of those factors is feeding schedule.[81] Also a time-restricted feeding schedule can change the rhythmic phase of locomotor activity, physiological function including corticosterone and clock genes in periphery by up to 12 h while leaving the rhythmic phase of clock genes in the SCN unaffected.[77,81] On the other hand, the manipulation of the feeding schedule can modify the chronopharmacological action and chronopharmacokinetics of drugs.[82] In humans, the pattern of diet intake substantially modifies plasma cortisol levels in addition to body temperature rhythm.[83] To produce new rhythmicity by manipulating the conditions of living organs by using rhythmic administration of altered feeding schedules or several drugs appears to lead to the new concept of chronopharmacotherapy.

CONCLUSIONS

The effectiveness and toxicity of many drugs vary depending on dosing time associated with 24 h rhythms of biochem-

ical, physiological and behavioral processes under control of the circadian clock. The knowledge of 24 h rhythm in the risk of disease plus evidence of 24 h rhythm dependencies of drug pharmacokinetics, effects, and safety constitutes the rationale for pharmacotherapy. From the viewpoint of pharmaceutics, the application of biological rhythm to pharmacotherapy may be accomplished by the appropriate timing of conventionally formulated tablets and capsules, and special drug delivery systems to synchronize drug concentrations to rhythms in disease activity. New technology for delivering medications precisely in a time-modulated fashion by bedside or ambulatory pumps is being developed to manage human diseases. In addition to chronopharmaceutical technology, the identification of a rhythmic marker for selecting dosing time will lead to improved progress and diffusion of chronopharmacotherapy. To monitor the rhythmic markers such as clock genes it may be useful to choose the most appropriate time of day for administration of drugs that may increase their therapeutic effects and/or reduce their side effects. Furthermore, to produce new rhythmicity by manipulating the conditions of living organs by using rhythmic administration of altered feeding schedules or several drugs appears to lead to the new concept of chronopharmacotherapy. Attention should be paid to the alteration of clock gene expression, 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. One approach to increasing the efficiency of pharmacotherapy is administering drugs at times during which they are best tolerated. Knowledge concerning clock genes should be applied to clinical practice. Therefore, the regulatory systems of biological rhythms should be clarified in detail from the viewpoint of clock genes.

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