Chapter 3

Circadian Clock Gene Expression and Drug/Toxicant Interactions as Novel Targets of Chronopharmacology and Chronotoxicology

Jie Liu, Huan Li, Shangfu Xu, Yunyan Xu and Chang Liu

Additional information is available at the end of the chapter

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Abstract

Circadian rhythms are driven and maintained by circadian clock gene networks in both brain and peripheral organs. In the liver, circadian rhythms produce oscillation in drug Phase-I, Phase-II, and Phase-III (transporters) metabolism genes, which in turn would affect drug disposition and detoxication, resulting in diurnal variations of efficacy and toxicity when drugs are given at different times of the day. On the other hand, drugs and toxicants could affect circadian clock gene expression to produce biological effects leading to therapeutic or toxic outcomes. This chapter reviewed the relevant literature and a dozen of publications from our work, discussed the interactions of circadian clock genes with drugs and/or toxicants to better understand the importance of circadian clock gene expression as novel targets in Pharmacology and Toxicology.

Keywords: circadian clock gene expression, liver, drug metabolism oscillation, chronopharmacology, chronotoxicology, brain

1. Introduction

Organisms on earth developed the ability to predict and restrict their activity to the night or day by endogenous circadian clock [1, 2]. The mammalian circadian clock system is timed to a 24-h solar time period and maintains rhythmic physiology. In mammals, the circadian clock influences nearly all aspects of physiology and behavior, including sleep-wake cycles, cardiovascular activity, endocrine function, body temperature, kidney function, physiology of the gastrointestinal tract, hepatic metabolism, immune function, detoxification, and the

reproductive system [3, 4]. Disruption of biological rhythms produces negative effects in the short and long terms leading to various diseases [4]. For example, clock dysfunction accelerates the development of liver diseases such as fatty liver diseases, hepatitis, cirrhosis, and liver cancer. Liver disorders also, in turn, disrupt circadian clock function [5].

Circadian oscillations are generated by a set of genes forming a transcriptional autoregulatory feedback loop. In mammals, these include the core clock regulators (Clock, Bmal1, and Npsa2), the clock feedback loop regulator genes (Per1, Per2, Per3, Cry1, and Cry2), and the clock target genes (DBP, Rev-erbα (Nr1d1), RORα, Tef, CK1δ, etc.) [6, 7]. The central clock is located in the suprachiasmatic nucleus in the hypothalamus and peripheral clocks in all tissues. Peripheral clocks in the liver have fundamental roles in maintaining liver homeostasis, including the regulation of energy metabolism and the expression of enzymes controlling the absorption and metabolism of xenobiotics [8]. Over the past three decades, researchers have investigated the molecular mechanisms using global clock-gene knockout mice, or clock gene mutant mice, or other genetic and molecular biology tools to elucidate molecular architecture of circadian clock in mammals [9].

Chronopharmacology and chronotoxicology is a new interdisciplinary science aimed at studying the influence of circadian system on drug disposition, efficacy, and toxicity. Xenobiotics absorption, distribution, metabolism, especially by P450, and excretion [10–14], all under circadian regulation. Circadian variations on these hepatic drug processing genes [15] greatly influence therapeutic effects and toxicity of drugs [10, 16–18]. The chronotherapy of anticancer drugs gives an excellent example [18]. This chapter will focus on the general aspects of circadian rhythms on drug/toxicant disposition and biological effects, and will also discuss the effects of drugs/toxicants on circadian clock gene expression as a novel target of chronopharmacology and chronotoxicology. A dozen of our publications in recent 5 years were also included for discussion.

2. Circadian rhythms affect Phase-I, Phase-II, and transporter gene expressions in the liver

Liver is the major site of xenobiotics metabolism and disposition. Accumulating evidence clearly indicates that circadian rhythms affect the gene/protein expression encoding xenobiotics uptake (Oatps and Ntcp), Phase-I metabolism (P450) and detoxication (Nrf2, MT-1, and GSH systems), Phase-II conjugation (glutathione S-transferases, UDP-glucuronosyltransferases, and sulfotransferases), and efflux transporters (Mrps and MDR) (**Figure 1**).

Diurnal variation of hepatic uptake transporters. In the liver, the major uptake transporters are organic anion transporting polypeptides (Oatp1a1, Oatp1a4, Oatp1b2, and Oatp2b1), organic cation transporter (Oct1), organic anion transporters (Oat2 and Oat6), and others [19]. The expressions of Oatp1a1, Oatp1a4, Oatp1b2, Oct1, and Oat2 display diurnal oscillations, with higher expression in the morning, while Oatp2b1 did not show circadian variation [20]. Na^+-taurocholate cotransporting polypeptide (Ntcp and Slc10a1) is a major bile acid uptake transporter that localizes to the basolateral membrane of hepatocytes, and displays apparent circadian rhythm, with higher expression in the afternoon [20–22].

Diurnal variation of hepatic Phase-I P450 metabolism enzyme genes. Hepatic cytochrome P450 is the major enzyme catalyzing the Phase-I drug metabolism. Most drugs are metabolized by

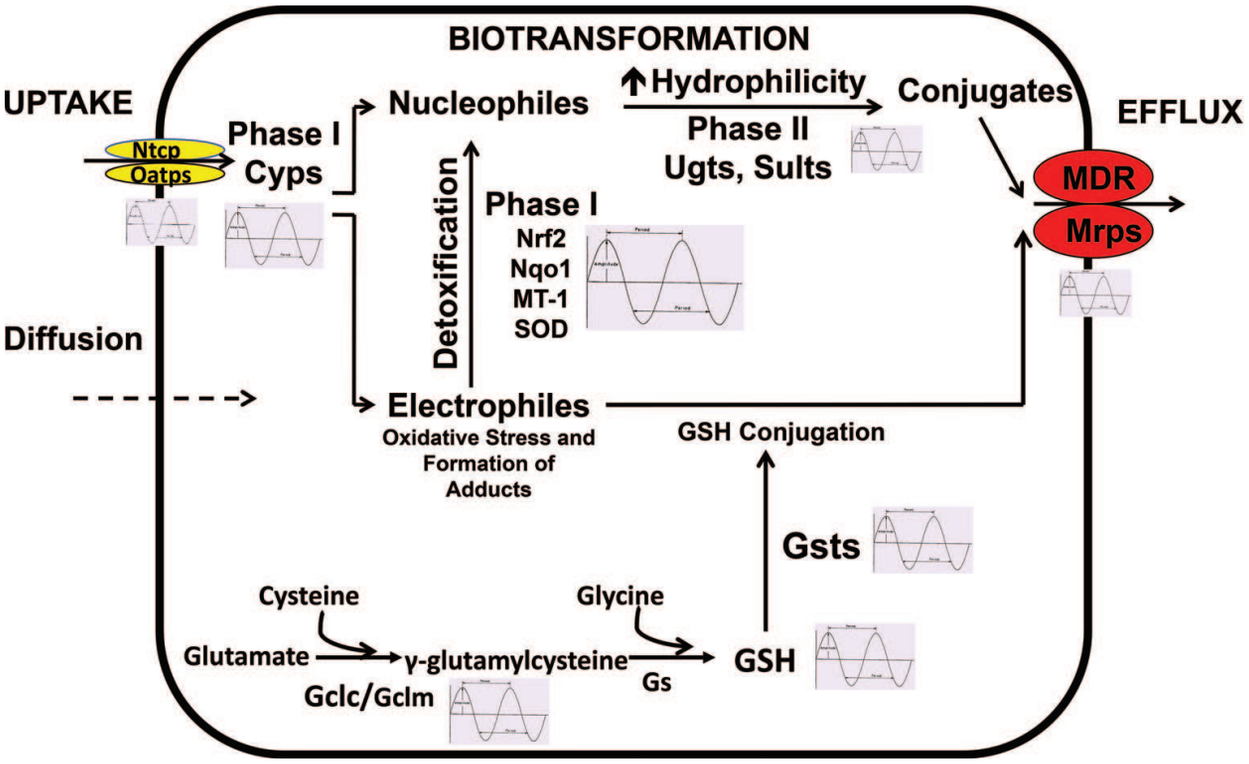

Figure 1. Drug metabolism (Phase-I, Phase-II, transporter) and detoxication (GSH, Nrf2, MT-1) gene expression show circadian oscillations.

P450 1–4 family enzymes. P450 enzyme genes and corresponding nuclear receptors display diurnal oscillations: AhR and Cyp1a1, 1a2 are higher in the morning; CAR and Cyp2b10 are higher in the afternoon and evening; PXR is higher in the afternoon but Cyp3a11 and Cyp3a25 are higher in the morning; PPARα is higher in the morning but Cyp4a10 is higher in the evening [23]. Cyp7a1 is a rate-limit enzyme gene for bile acid synthesis, displays a typical circadian rhyme, with the peak around 18:00 [21–24]. Bile acid synthesis is controlled by the circadian clock and Rev-erbα is a major clock gene controlling bile acid homeostasis [25].

In the liver, circadian rhythm serves to synchronize the metabolism of bile acid, glucose, and lipid, and their disruption could lead to diseases and affect chronotherapy [26]. Indeed, the liver is the key organ to maintain energy metabolism which is greatly influenced by feeding, diets, and diurnal variation [5]. For example, Peroxisome proliferator-activated receptor-gamma coactivator (PGC1α) stimulates the expression of clock genes, notably Bmal1 (also called Arntl) and Rev-erbα (also called Nr1d1), through coactivation of the ROR family of orphan nuclear receptors. Mice lacking PGC-1α show abnormal diurnal rhythms of activity, body temperature and metabolic rate [27]. Circadian clocks regulate metabolic processes not only by simply in response to daily environmental/behavioral influences but also by synchronizing the cell with its environment to modulate a host of metabolic processes [27–29].

Diurnal variation of hepatic detoxification enzyme genes. Many antioxidant enzyme genes display diurnal variations, such as the Nrf2 detoxication pathway genes [30], enzymatic detoxication components such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px1) and non-enzymatic protein such as metallothionein (MT) [31]. GSH is low in the afternoon which is partially responsible for acetaminophen hepatotoxicity when given in the afternoon [23].

Diurnal variation of hepatic Phase-II metabolism gene/proteins. Glucuronide and sulfate conjugations are major Phase-II pathways in the biotransformation and elimination of a wide variety of endogenous compounds, drugs, and other xenobiotics. Diurnal variations of these Phase-II reactions were reported in the 1980s [32]. Consistent to the variation in the conjugation reactions, the expression of Ugt1a5, 2a3, 2b34, 2b36 and UDP-gpb, as well as Sult1a1, 1a5, and Sult5a1, all show diurnal oscillations [20]. Hepatic GSH has the trough at dusk [30], and the activities of GSH S-transferase [33] were lower at the dark phase and the expression of Gst1a1/1, Gst1a4, Gstm2, and Gstt1/2 display diurnal rhythms which are generally lower in the dark phase [20].

Diurnal variation of hepatic Phase-III efflux transporters. P-glycoprotein is the major efflux pump in the liver, and its expression shows circadian variation together with the diurnal expression of Abcb1 [34]. In addition to P-glycoprotein, hepatic multidrug-resistant protein 2 (MRP2), breast cancer resistant protein (BCRP) also show circadian oscillations [35]. Diurnal variations in hepatic mRNA expression of multidrug-resistant gene 1a (Mdr1a), Mrp2, and Bcrp were also evident [20, 35].

Diurnal variation of hepatic Phase-I, Phase-II, Phase-III, and the nuclear transcription factors would affect the xenobiotic metabolism when administered at the different times of the day to impact their efficacy and toxicity, the time really matters [15].

3. Circadian rhythm disruption affects therapeutic effects and toxicity of xenobiotics in the liver

Table 1 gives a few examples of how the disruption of circadian clock could affect drug effect and toxicity. Most of the examples used genetic models with disruption of circadian clock genes or administration of drugs at different times.

Carbon tetrachloride is a commonly used hepatotoxicant. In SD rats, administration of CCl_4 in the afternoon showed more toxicity than administrated in the morning, the increased toxicity was accompanied by the lowest hepatic GSH levels in the afternoon [36]. Acute CCl_4 toxicity was increased in Per2−/− mice. At the 12-h time point after CCl_4 treatment, more vacuolations were observed in the liver tissues of Per2-null mice as compared to wild-type (WT) mice, and at 24 h after CCl_4 treatment, more severe hepatic necrosis was evident than that occurred in WT mice. A deficit of the Per2 gene enhanced Ucp2 gene expression levels in the liver leading to reduced ATP and increased production of toxic CCl_4 derivatives. The absence of Per2 also caused an increased expression of Clock gene [37]. Per2-null mice were not only sensitive to CCl_4-induced acute hepatotoxicity, but also to CCl_4-induced chronic toxicity and fibrosis. CCl_4 caused much more severe liver fibrosis and activated hepatic stellate cell (HSC) in mPer2 null mice as compared to WT mice. Per2-null mice exhibited less efficiency in fibrosis resolution and apoptosis resistance in HSC. Transfection of Per2 cDNA into CCl_4-exposed HSC restored apoptosis sensitivity with up-regulation of the TRAIL-R2/DR5 signaling pathway [38].

Acetaminophen hepatotoxicity also displays diurnal variations. When given acetaminophen in the afternoon, toxicity was greater than that given in the early morning [23, 39]. At 8:00, there

Drug/toxicant	Animal models	Chronotoxicology	References
Carbon tetrachloride	SD rats	18:00 toxicity >6:00, with lowest GSH levels	[36]
Carbon tetrachloride	Per2−/− mice	Acute toxicity increased in Per2−/− mice	[37]
Carbon tetrachloride	Per2−/− mice	Chronic toxicity, fibrosis increased in Per2−/− mice	[38]
Acetaminophen	KM mice	18:00 toxicity >6:00	[23]
Acetaminophen	Per2−/− mice	Toxicity decreased in Per2−/− mice	[40]
Acetaminophen	Clock−/− mice	Toxicity decreased in Per2−/− mice, with prolonged PBST	[41]
Acetaminophen	$Bmal1^{fx/fx}Cre^{Alb}$ mice	Reduced toxicity, reduced protein adducts, altered APAP metabolism	[42]
Dixon (TCDD)	$Per1^{Idc}$,$Per2^{Idc}$ mice, cells	Increased TCDD induction of Cyp1a1, Cyp1b1	[43]
Dixon (TCDD)	$Per1^{Idc}$,$Per2^{Idc}$, Per1/$Per2^{Idc}$ mice	Abolished diurnal variation of TCDD induction of Cyp1a1	[44]
Benzo[a]pyrene	Clock mutant (Clk/Clk) mice	Abolished diurnal variation of B[a]P induction of Cyp1a1	[45]
Bile duct ligation	Per2−/− mice	Increased BDL-induced liver injury and fibrosis	[46]
Cholestyramine diet restricted feeding	$Per1^{-/-}$/$Per2^{-/-}$ mice	Lost diurnal variation in bile acid metabolic enzyme genes	[47]
Isoniazide	Swiss mice	Isoniazid hepatotoxicity at ZT1 > ZT9, ZT17	[48]
Chlorozoxazone	Wistar rats	Diurnal variation in CYP2E1 affect its half-life	[49]
Alcohol	Per1−/−, Per2−/− mice	Less susceptible to alcohol toxicity	[50]
Diethylnitrosamine (DEN)	$Clock^{mut}$ mouse hepatocytes	Decreased DEN metabolism and apoptosis tolerance	[51]
Cadmium	ICR mice	Toxicity at ZT 8 > ZT 20, corresponding to low level of GSH at ZT8	[52]

Table 1. Circadian clock gene expression as novel targets in toxicology.

was no difference of acetaminophen toxicity between Per2-null and WT mice, but at 20:00 when the Per2 expression is highest, Per2-null mice had less liver injury, with less Cyp1a2 expression to bio-activate acetaminophen [40]. In another study, acetaminophen toxicity is greater at Zeitgeber time (ZT)14 than at ZT2, and clock-deficient mice are resistant to the toxicity at ZT14, with prolonged pentobarbital sleep time (PBST), indicating the reduced activation of acetaminophen [41]. Use Bmal1 mutant mice ($Bmal1^{fx/fx}Cre^{Alb}$), the acetaminophen toxicity at ZT12 was decreased, along with decreased APAP protein adducts and altered acetaminophen metabolism kinetics (increased AA-Gluc), possibly due to decreased NADPH-cytochrome P450 oxidoreductase gene expression and activity at ZT12, as compared to WT mice [42].

In Per1, Per2-deficient mice, the ability of AhR ligand dioxin (TCDD) to induce the Cyp1a1 and Cyp1b1 was enhanced, especially with targeted interruption of Per1 [43]. TCDD induction of Cyp1a1 was 23–43 fold greater during the night time (ZT18) than at the day time (ZT6) in WT mice. However, the diurnal variation in the TCDD induction of Cyp1a1 expression was abolished in $Per1^{ldc}$, $Per2^{ldc}$, and $Per1^{ldc}/Per2^{ldc}$ mutant mice, suggesting that Per1, Per2 and their timekeeping function in the circadian clockworks mediate the diurnal variation in TCDD induction of Cyp1a1 [44]. Clock mutant Clk/Clk mice failed to show typical oscillation of AhR expression, and BaP (an AhR ligand) induction of Cyp1a1 was disrupted [45].

In Per2−/− mice, bile duct ligation (BDL)-induced liver injury and fibrosis was increased, along with increases in TNFα, TGFβ1, Col1α, and TIMP1 in livers of Per2-null mice as compared to WT mice [46]. In Per1−/− and Per2−/− mice fed on 2% cholestyramine diet, and/or restricted feeding (phase-shift peripheral clock), liver bile acid levels were increased, and the nuclear receptors CAR and PXR were activated, together with the increased serum enzyme AST levels, indicative of liver damage. In these Per1−/− and Per2−/− mice, the circadian expression of key bile acid synthesis and transport genes, including Cyp7a1 and Ntcp, was lost [47].

The hepatotoxic potential of antituberculosis drug isoniazid varied when it was administered at ZT1, ZT9, and ZT17, and the toxicity was highest when isoniazid was given at ZT1 [48]. Chlorzoxazone is a CYP2E1 metabolized drug, and its kinetics and half-life were altered with the diurnal variation of CYP2E1 activity. The value of chlorzoxazone half-life in plasma of the light phase group was significantly longer than the dark phase group, with an increase of 6-hydroxychlorzoxazone production [49]. Acute alcohol-induced higher toxicity at ZT13 than ZT1 when Per1 and Per2 were highly expressed. Per1−/− and Per2−/− mice were less susceptible to alcohol hepatotoxicity, especially in Per1 null mice. Per1 null mice had decreased expression of peroxisome proliferators-activated receptor-gamma and its target genes related to lipid metabolism such as Srebp1, fatty acid synthase (Fas), CD36, diacylglycerol O-acyltransferase 2 (Dgat2), AP2, and adipsin [50]. In primary hepatocytes isolated from Clock mutant Clk/Clk mice and WT mice, diethylnitrosamine (DEN) induced apoptosis and cell death were reduced in Clock-deficient mice, probably due to decreased DEN metabolism [51]. Cadmium hepatotoxicity is independent of metabolic activation; while its mortality was high at ZT8 than ZT20 when the hepatic GSH level was lowest [52].

Thus, alterations of diurnal oscillations would affect drug metabolism, efficacy, and toxicity. On the other hand, drugs could target circadian clock gene expressions to produce biological effects, which will be discussed below.

4. Drugs/toxicants could affect both central and peripheral circadian clock gene expression

The circadian clock is located in both brain and peripheral tissues [8]. The central clock pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, while the peripheral clock is distributed in all peripheral tissues. The liver is the main peripheral tissue under circadian clock regulation [7–9]. Drugs/toxicants could affect both central and peripheral clock gene expression. For example, Mn is a well-known neurotoxicant

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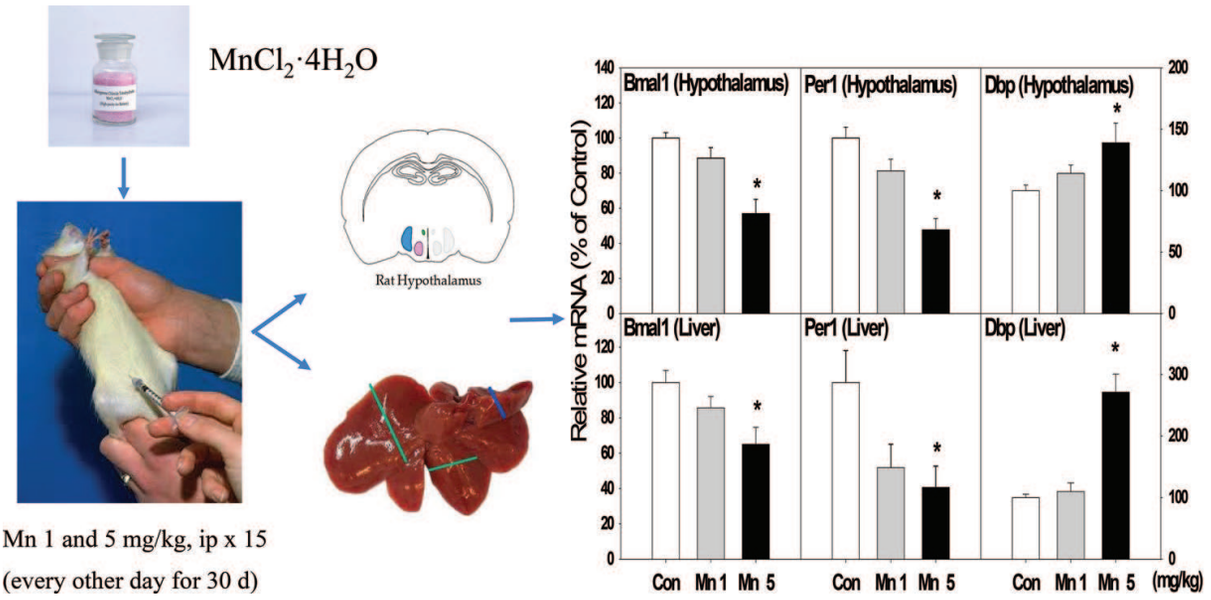

Figure 2. Neurotoxicant manganese intoxication produced aberrant expression of circadian clock genes in both central (hypothalamus) and peripheral (liver). Adapted from Li et al. [53].

producing a Parkinson-like syndrome, but it also produces liver injury. In an attempt to examine the effect of Mn on the central and peripheral clocks, rats were given Mn 1 and 5 mg/kg, ip, every 2 days for 1 month, and the hypothalamus and liver were removed to examine the clock gene expression (**Figure 2**). The results showed that Mn-induced aberrant expression of circadian clock genes in both hypothalamus and liver, and liver was more sensitive to Mn-induced decreases in clock gene Bmal1, Per1, and increase in Dbp, indicating that both central and peripheral clocks could be disrupted by drugs/toxicants [53]. Another example is chronic alcohol administration. Chronic alcohol consumption produced disruption of circadian clock gene expression in both central (hypothalamus) and peripheral tissues (liver and colon) [54], and the liver appeared to be more susceptible than brain in alterations of metabolic genes and core molecular clock disruption. In addition to the fatty liver and affected the diurnal oscillations of metabolic genes (alcohol dehydrogenase 1, carnitine palmitoyltransferase 1a, Cyp2e1, Phosphoenolpyruvate carboxykinase 1, pyruvate dehydrogenase kinase 4, Ppargc1a, Ppargc1b and Srebp1c), the diurnal oscillations of core clock genes (Bmal1, Clock, Cry1, Cry2, Per1, and Per2) and clock-controlled genes (Dbp, Hlf, Nocturnin, Npas2, Rev-erbα, and Tef) were altered in livers from ethanol-fed mice. In contrast, ethanol had only minor effects on the expression of core clock genes in the suprachiasmatic nucleus (SCN) [55].

5. Drugs affect circadian clock gene expression as a novel target of chronopharmacology

Many drugs/toxicants could affect central and peripheral circadian clock gene expression as targets of chronopharmacology and chronotoxicology [10]. **Tables 2** and **3** provide some examples including our work in the field.

Drugs	Animal model (dose, route, time)	Chronopharmacology	References
Atorvastatin	KM mice; 10–100 mg/kg, po × 30 days	Swollen hepatocyte and feather-like degeneration; increased Cyp7a1, FXR, decreased bile acid transporters; increased expression of Bmal1, Npas2, decrease Per2, Dbp.	[56]
Metformin	C57 mice; 164 mg/kg in drinking water for 6 weeks	Increase in serum leptin and decreased glucagon levels. Increase in PGC1α, PPARα, AMPK; decrease in ACC in liver; Phase advance circadian clock and metabolic genes in liver and activation of liver casein kinase Iα (CKIα)	[58]
Oleanolic acid	Apoe−/− mice on HFD, F344 rats; 0.01% OA × 11 weeks	Increased lipid droplets with no change in oxidative stress; increased Bmal1, Clock, and Elovl3, Tubb2a, and Cldn1 decreased Per3, Amy2a5, Usp2, and Thrsp.	[59]
Resveratrol	C57 mice; fed normal or HFD; 0.1% Res × 11 weeks	Ameliorated HFD-increased plasma leptin, lipids, and BW. Restored rhythmicity of Clock, Bmal1, and Per2; and clock-controlled lipid metabolism genes (Sirt1, PPARa, Srebp-1, Acc1, and Fas).	[60]
Sea cucumber saponin (SCS)	ICR mice; 0.03% SCS diet night feeding × 2 weeks	Improve serum lipid profile; restore rhythmicity of PPARα, Srebp1, Cpt, and FAS; restore nighttime feeding-disrupted clock gene expression.	[61]
Zuotai	KM mice; 10 mg/kg, po × 7 days	Decreased the amplitude of Clock, Npas2, Bmal1; increased Dbp, Nfil3 at 10:00, and increased Nr1d1 at 18:00. No effect on Cry and Per genes.	[62]
Polyporus and Bupleuri radix	ICR mice, $Per2^{Luc}$ mice 500 mg/kg, po × 3 days, at different ZT and light/dark	Polyporus and Bupleuri radix were effective in manipulating the peripheral circadian clock phase acutely, with stimulation time-of-day dependency in vitro as well as in vivo.	[63]
Jiao-Tai-Wan	SD normal and model (HFD + PSD × 4 weeks) rats 2.2 g/kg, po × 4 weeks	Increased total sleep time and slow wave sleep time; reversed model rat-induced inflammation markers; increased Cry1, Cry 2, and decreased NF-κB in PBMC.	[64]

Table 2. Circadian clock gene expression as novel targets in pharmacology.

Examples of drugs Atorvastatin is an HMG-CoA reductase inhibitor used for hyperlipidemia. It is generally safe but may induce cholestasis. Repeated administration of Atorvastatin (10–100 mg/kg, po) to mice for 30 days produced hepatocyte swollen and feather-like degeneration, indicative of cholestatic injury, with increases of inflammation markers Egr1 and MT-1, and increased Cyp7a1, FXR, SHP, decreased bile acid transporters Ntco, Bsep, Oastα, and Ostβ. Since Cyp7a1 is a clock-driven gene, its effects on circadian clock gene expression were also examined. Atorvastatin increased the expression of Bmal1, Npas2, decreased the expression of Per2, Per3, Dbp, and Tef, but had no effect on Cry1 and Nr1d1 [56]. The similar effects on the circadian clock gene expression were also observed when atorvastatin was given at the low dose (10 mg/kg) but for a longer period of 90 days, although to a less extent [57].

Metformin is commonly used for type 2 diabetes. In C57 mice, metformin in the drinking water for 6 weeks led to increased serum leptin and decreased glucagon levels. The effect of metformin on liver and muscle metabolism was probably mediated through AMPK activation,

	Animal model (dose, route, time)	Chronotoxicology	References
Carbon tetrachloride	BABL/C mice 0.6 ml/kg, ip, 2/week × 4 weeks	Chronic CCl_4 produced liver fibrosis, altered the amplitudes, meros, acrophases of clock gene expression; circadian rhythms of Cry2, PPARα and POR were lost.	[65]
Diethylnitrosamine	KM mice DEN 100 mg/kg, IP+ CCl_4 + EtOH × 16 weeks	Produced HCC, Markedly increased α-fetoprotein; at 10:00, expression of Bmal1 decreased, expressions of Dbp and Rev-erbα increased.	[66]
Manganese	SD rats 1 and 5 mg/kg, IP, × 4 weeks	Produced neuroinflammation and dopaminergic neuron loss; decreased expression of Bmal1, clock, Per1, Per2, while increased expression of Dbp and Nr1d1	[53]
LPS + Rotenone	SD rats LPS 5 mg/kg, IP ×1, 200 days later, rotenone 0.5 mg/kg, sc × 20	Produced neuroinflammation and dopaminergic neuron loss; at the mRNA and protein levels, reduced expression of Bmal1, clock, Per1, Per2, Dbp, Nr1d1, while no effect on Cy1.	[70]
LPS	ICR mice, LPS 1 mg/kg, IP at ZT4, 10, 16, 22 or at 2, 8, and 26 h after ZT 4 injection	Produced increases in serum TNFa, heart and liver apoptosis; Decrease Per1, Per2 2 h after dose at ZT4 in heart and liver; Increased Per2 8 and 26 h after LPS in heart and liver	[71]
Alcohol	C57 mice, $Per2^{Luc}$ mice Lieber-DeCarli diet for 30–37 days	Produced steatosis, increased serum TG; diurnal oscillations of Bmal1, Clock, Cry1, Cry2, Per1, and Per2 and clock-controlled genes (Dbp, Hlf, Nocturnin, Npas2, Rev-erba, and Tef) were altered in livers of ethanol-fed mice	[55]
Alcohol	WT and $Clock^{\Delta 19}$ mutant mice received Nanji liquid alcohol diet at ZT4 for 10 weeks	Altered the expression of circadian and metabolism genes in hippocampus, liver, and colon from array analysis; $Clock^{\Delta 19}$ affect inflammation and metabolism gene.	[54]

Table 3. Circadian clock gene expression as novel targets in toxicology.

resulting in the inhibition of acetyl CoA carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis. Metformin-activated liver casein kinase I α (CKIα) and muscle CKIε, known modulators of the positive loop of the circadian clock, thud resulting in phase advances in the liver and phase delays in the muscle for clock and metabolic gene expressions [58].

Examples of active ingredients from herbal medicine. Oleanolic acid is a triterpenoid used to reduce hyperlipidemia. Dietary oleanolic acid supplementation (0.01%) was provided to Apoe- and Apoa1-deficient mice and F344 rats. In Apoe-deficient mice, oleanolic acid supplementation increased hepatic lipid droplets, increased circadian clock genes, together with increases in lipid metabolism genes (fatty acid elongase 3, tubulin beta-2A chain, and claudin 1), while the expression of per3, amylase 2a5, ubiquitin-specific peptidase 2, and thyroid hormone-inducible hepatic protein (Thrsp) were decreased [59].

Resveratrol is an active ingredient in grapes and red wine and shows beneficial effects in metabolic disorders. In HFD-fed mice, resveratrol restored high-fat diet-induced disorders about

the rhythmic expression of clock genes and clock-controlled lipid metabolism, ameliorated the rhythmites of plasma leptin, lipid profiles and whole body metabolic status (respiratory exchange ratio, locomotor activity, and heat production). Meanwhile, resveratrol modified the rhythmic expression of clock genes (Clock, Bmal1, and Per2) and clock-controlled lipid metabolism-related genes (Sirt1, Ppara, Srebp-1c, Acc1, and Fas) [60].

Dietary sea cucumber saponin (SCS) has been shown to have beneficial effects on glucose and lipid metabolism, which is related to the circadian clock. Dietary SCS caused an alteration in rhythms and/or amplitudes of clock genes was more significant in the brain than in liver. In addition, the peroxisome proliferator-activated receptor (PPARα), sterol regulatory element binding protein-1c (SREBP-1c), together with their target genes carnitine palmitoyl transferase, and fatty acid synthase showed marked changes in rhythm and/or amplitude in SCS group mice [61].

Examples of mixtures from traditional medicine. Zuotai is an essential component of many popular Tibetan medicines. Mice were orally given Zuotai (10 mg/kg, 1.5-fold of clinical dose) daily for 7 days, and livers were collected every 4 h during the 24 h period to examine its effects on circadian clock gene expression. Zuotai decreased the oscillation amplitude of Clock, Npas2, Bmal1 at 10:00. For the clock feedback negative control genes, Zuotai had no effect on the oscillation of Cry1, Per1, Per2, and Per3. For the clock-driven target genes, Zuotai increased the oscillation amplitude of Dbp, decreased nuclear factor interleukin 3 (Nfil3) at 10:00, but had no effect on thyrotroph embryonic factor (Tef); Zuotai increased the expression of Nr1d1 at 18:00, but had little influence on Nr1d2 and RORα [62].

Polyporus and Bupleuri radix were popular traditional medicines. Polyporus (Zhulin) is used as a diuresis in the treatment edema, while Bupleuri radix (Chaihu) is used for chronic hepatitis. The $Per2^{Luc}$ mice were used to screen their effects on the circadian clock, and Polyporus was more effective than Bupleuri radix in manipulating the peripheral circadian clock phase-shift, and in promoting time-of-day dependency in vitro as well as in vivo [63].

Jiao-Tai-Wan (JTW), composed of Rhizome Coptidis and Cortex Cinnamomi, is a classical traditional Chinese prescription for insomnia. In obesity-resistant (OR) rats with chronic partial sleep deprivation (PSD) model, 4 weeks of administration of JTW increased total sleep time and total slow wave sleep (SWS) time in OR rats with PSD, and reversed the mode rats elevated serum markers of inflammation and insulin resistance, and these changes were also associated with the up-regulation of Cry1 mRNA and Cry 2 mRNA and the down-regulation of NF-κB mRNA expression in peripheral blood monocyte cells [64].

6. Toxicants affect circadian clock gene expression as a novel target of chronotoxicology

Table 3 lists some examples of known toxicants which disrupted circadian clock gene expression as a mechanism of their acute and chronic toxic effects to both brain and liver.

Examples of hepatotoxicants. Chronic carbon tetrachloride administration in C57 mice (0.6 mL/kg, IP, twice a week for 4 weeks) produced liver injury and fibrosis. The expression of clock genes and metabolic genes in fibrosis livers was altered. The amplitudes of circadian expressions of

Bmal1 and Per1 were attenuated and the mesors in the expressions of Clock and Per1 were increased. Acrophases for the expressions of Clock, Per1 and Cry1 were significantly delayed. Circadian rhythm of Cry2 expression was lost in fibrosis group. The circadian rhythm of PPARα and cytochrome P450 oxidoreductase (POR) was also lost [65].

Chronic diethylenediamine (DEN) administration not only produce hepatocellular carcinoma and markedly enhanced expression of Afp, but also decreased the expression of Bmal1, increased the expression of Dbp and Rev-erbα (Nr1d1) [66]. Circadian disruption is well-known to promote carcinogenesis [67]. In the end-stage of human hepatocellular carcinoma, the expressions of the clock genes, including Bmal1, Per1, Per2, Cry1, and Cry2 were decreased, alone with decreases in clock targeted MT-1, MT-2, and MTF1 (which are considered as biomarkers of HCC). On the other hand, the expression of clock target genes Nr1d1 and Dbp was upregulated as compared with Peri-HCC and normal livers. Peri-HCC also had mild alterations in these gene expressions [68].

Examples of neurotoxicants. As mentioned in **Figure 2**, repeated Mn administration disrupted both central and peripheral liver circadian clock genes, with decreases in Bmal1, Clock, Npas2, Per1, Cry1, but increases in Dbp and Nr1d1. Mn-induced aberrant expression of these clock genes in the brain was consistent with that in the liver, and liver appeared to be more sensitive than hypothalamus to Mn-induced disruption of circadian clock [53].

Chronic neuroinflammation would aggregate neurotoxic effects of toxicants. Rats received a single injection of LPS at the dose of 5 mg/kg, and 200 days later given repeated injection of low dose of rotenone (0.5 mg/kg, sc, 5/week for 4 weeks), and produced neuroinflammation and loss of dopaminergic neurons in Substantia Nigra, replicate the model of Parkinson's disease [69]. In this PD model, aberrant expression of circadian clock genes in brain cortex was evident, as evidenced by decreases of core clock gene Bmal1, clock, and Naps2, decreases in circadian clock feedback gene Per1 and Per2, but had no effect on the expression of Cry1 and Cry2, as well as the decreased expression of clock target gene Dbp and Nr1d1 [70].

LPS not only produces inflammation in the brain but also in the liver. ICR mice received LPS (1 mg/kg, IP) at ZT4, ZT10, ZT16, and ZT22, and liver and heart were harvested 2 h later for gene expression analysis. Hepatic expression of Per1 and Per2 was decreased after LPS injection at ZT6, but Per1 was increased 8 and 26 h after LPS injection. Heart speared to be more sensitive than the liver to these changes as at ZT4, both Per1 and Per 2 in the heart were decreased [71].

Examples of chronic ethanol toxicity. Alcoholic liver diseases are a major concern as it produced metabolic disruption. In C57 mice and Per2 mutant mice, ethanol administration altered the expression of clock genes in the liver, but not in the brain. Diurnal oscillations of core clock genes (Bmal1, Clock, Cry1, Cry2, Per1, and Per2) and clock-controlled genes (Dbp, Hlf, Nocturnin, Npas2, Rev-erba, and Tef) were altered in livers from ethanol-fed mice [55].

In clock mutant mice, altered clock and metabolism genes were evident in hippocampus, liver, and colon. Of particular interest was the finding that a high proportion of genes involved in inflammation and metabolism on the array was significantly affected by alcohol and the Clock gene mutation in the hippocampus [54].

Thus, drugs/toxicants could affect central and peripheral circadian clock gene expression as targets of their therapeutic effects and/or toxicity [10].

7. Summary and perspectives

The importance of chronopharmacology has been reviewed 10 years ago [16]. Circadian rhyme governs many physiological functions, and the RNA-Seq revealed that over 3000 genes in the liver showed circadian oscillation [72]. Over the past two decades, research has investigated the molecular mechanisms linking circadian clock genes with the regulation of hepatic physiological functions, using global clock-gene-knockout mice, or mice with liver-specific knockout of clock genes or clock-controlled genes. Clock dysfunction accelerates the development of liver diseases such as fatty liver diseases, cirrhosis, hepatitis, and liver cancer, and these disorders also disrupt clock function. Similarly, clock dysfunction clearly affects drug efficacy and toxicity.

In the liver, Phase-I is composed mainly of cytochromes P450 involved in detoxification and hormone and lipid metabolism [11], which are regulated by nuclear receptors. Phase-II enzymes modify the phase-I metabolites by conjugation reactions, while phase-III includes membrane transporters responsible for the elimination of modified xenobiotics. Phases I–III of drug metabolism are under strong circadian regulation [15]. The rhythmic control of xenobiotic detoxification provides the molecular basis for the dose- and time-dependence of drug toxicities and efficacy, and makes the circadian clock gene expression as a target for chronopharmacology [10], not only for drugs but also for traditional medicines [73]. Circadian rhythms also greatly affect drug toxicity at the different times of administration [74]. Circadian rhythms are controlled, regulated and maintained by clock gene networks, which are the emerging targets of chronopharmacology and chronotoxicology.

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Conflict of interest

The authors do not have conflict of interest.

Author details

Jie Liu[1]*, Huan Li[1,2], Shangfu Xu[1], Yunyan Xu[1] and Chang Liu[2]

*Address all correspondence to: jie@liuonline.com

1 The Key Lab for Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine, Zunyi Medical University, Zunyi, Guizhou, China

2 Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China

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