Review

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Research progresses on the effects of heavy metals on the circadian clock system

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Abstract: Environmental pollution with heavy metals is widespread, thus increasing attention has been paid to their toxic effects. Recent studies have suggested that heavy metals may influence the expression of circadian clock genes. Almost all organs and tissues exhibit circadian rhythms. The normal circadian rhythm of an organism is maintained by the central and peripheral circadian clock. Thus, circadian rhythm disorders perturb normal physiological processes. Here, we review the effects of heavy metals, including manganese, copper, cadmium, and lead, on four core circadian clock genes, i.e., *ARNTL*, *CLOCK*, *PER*, and *CRY* genes.

Keywords: circadian clock gene; circadian rhythms; heavy metals

Introduction

Affected by the rotation of the Earth, almost all biological activities adhere in some way to a cycle of approximately 24 h, referred to as the circadian rhythm [1]. Many physiological processes in mammals show characteristics of circadian rhythms, such as hormone secretion, heart rate, and body temperature regulation [2]. The suprachiasmatic nucleus (SCN) receives direct light information from the retina and thus is synchronized to the day/light cycle. Therefore, the SCN is assumed to be the control center of circadian rhythms and called the circadian clock pacemaker [3]. The SCN expresses a set of core circadian genes, including *CLOCK*, *ARNTL* (also known as *BMAL1*), *CRYs* (*CRY1/2*), and *PERs* (*PER1/2/3*), which together are termed the central clock system, maintaining normal physiological functions of the organism. Apart from the SCN, these genes are also expressed in various peripheral organs, where they are referred to as the peripheral clock system [4]. In physiological states, the central clock system governs peripheral clocks and synchronizes its oscillations. However, photoperiod changes and environmental factors can lead to circadian rhythmic disorders that cause diseases [5]. Heavy metals, such as manganese (Mn), cadmium (Cd), and lead (Pb), function as endocrine disruptors and can exert harmful effects on the clock systems. This review introduces the composition and function of the circadian clock system and the influence of heavy metals on this system.

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Literature search process

This review focuses on the relationship between the circadian rhythm and heavy metals. We searched relevant published studies using PubMed and Google Scholar, mainly with the following search terms: circadian rhythm, circadian rhythm gene, clock gene, arntl gene, per gene, cry gene, heavy metals, cadmium, copper, manganese, lead, endocrine system and endocrine hormones. The term AND was also used to identify highly relevant articles. In addition, references in relevant articles were examined for additional information. The titles and abstracts of all retrieved articles were carefully reviewed, and irrelevant and duplicate documents were eliminated.

The circadian clock system

In 2017, Jeffrey C. Hall, Michael Rosbash, and Michael W. Young were awarded the Nobel Prize in Physiology or Medicine for elucidating the genetic makeup and function of the circadian clock system [6]. The circadian clock is of great significance to human beings, and elucidating the relationship between the circadian clock and human diseases may inspire novel strategies for therapy and for improving the

patient's quality of life. The circadian clock is comprised of a central circadian clock located in the SCN and peripheral circadian clocks in various peripheral organs [7]. In mammals, molecular circadian clocks have four core circadian genes, transcription-translation feedback loop elicited by these core clock genes control the circadian rhythm of the body [8]. *CLOCK* and *ARNTL* form heterodimers that bind to E-box elements on *PER* and *CRY* genes to activate their transcription. The PER and CRY proteins accumulate and form dimers. At night, after PER/CRY dimer accumulation in the cytoplasm, they enter the nucleus and inhibit the synthesis of *CLOCK*/ARNTL heterodimers, thereby repressing their transcription, which is a transcription-translation feedback loop, the entire process takes approximately 24 h [9–11]. In addition to the core transcription-translation feedback loop, other auxiliary feedback loops are involved in circadian rhythm regulation, such as retinoic acid receptor-related orphan nuclear receptors (*ROR*) *α*, β, and γ and the Nuclear receptor *REV-ERBα* [12], REV-ERBα and β binds to the *Arntl* promoter and inhibits *Arntl* transcription, whereas RORα, β, and γ promotes *Arntl* transcription [13]. The circadian clock and the endocrine system are intimately linked, and several hormones regulate the circadian clock [14]. Glucocorticoids are important factors for the stability of the central and peripheral clocks, and high or low levels of glucocorticoids can lead to the abnormal expression of clock genes [15, 16]. Melatonin secreted by the pineal gland can restore the body's circadian rhythm [17]. Mice with diethylnitrosamine-induced hepatocellular carcinoma exhibited increases in the expression of *Clock* and *Arntl* in the liver and decreases in the expression of *Cry1*, *Per1*, *Per2*, and *Per3*, and melatonin intervention prevented these adverse changes [18].

CLOCK gene

The *CLOCK* gene plays a central role in regulating circadian rhythms and is central to the entire transcription-translation feedback loop, which is expressed in organs and tissues. Mutations in the *CLOCK* gene can cause circadian rhythm disorders in mammals, resulting in tumors and diseases [19, 20]. Therefore, the *CLOCK* gene is important for the regulation of physiological functions. Roybal et al. [21] induced *Clock* gene mutations in mice by *N*-ethyl-*N*-nitrosourea administration and found that *Clock*-mutant mice exhibited manic behavior resembling the manic state of human patients with bipolar disorder. Johansson et al. [22] evaluated the circadian expression of *CLOCK* and several other circadian clock-related genes in patients with schizophrenia and found that *CLOCK* mRNA levels in the patients' mononuclear blood cells were lower compared to those in the normal control group, suggesting that the *CLOCK* gene may be linked to the development of mental illness. The *Clock* gene also plays a vital role in maintaining the rhythm of metabolism, and mice with *Clock* gene mutations suffer from metabolic diseases such as hypercholesterolemia and hyperglycemia [23].

ARNTL gene

ARNTL is an important transcription factor that regulates the body's circadian rhythm [24]. A study showed that *Arntl* maintained the normal rhythm of blood pressure in the body; mice lacking *Arntl* in smooth muscle tissue showed a lower blood pressure and higher pulse pressure, and no circadian rhythm [25]. *ARNTL* also regulates lipid metabolism. A study has shown that *Arntl* is an important regulator of insulin-induced fat synthesis and the lack of *Arntl* inhibits the expression of lipogenic genes in the liver and reduces *de novo* lipogenesis in the liver [26]. In addition, in a model of colorectal cancer, through *Arntl* mutation-induced tumorigenesis, deletion of *Arntl* in the intestine increased tumor initiation and the number of tumors [27]. A study on the role of *ARNTL* in pancreatic cancer showed that it was an anti-oncogene in pancreatic cancer, and overexpression of *ARNTL* can inhibit the proliferation of cancer cells via the arntl-p53-Bcl-2 axis [28]; *ARNTL* binds to the promoter region of the p53 gene and thus activates p53, subsequently inhibiting its downstream target Bcl-2 to promote apoptosis [28].

The mammalian reproductive system is also regulated by the circadian clock [29]. Male *Arntl* knockout mice are sterile and show decreased testosterone levels and increased concentrations of luteinizing, suggesting that the decreased expression of acute regulatory protein synthesis of steroid hormones caused by *Arntl* knockout may be the cause of reproductive disorders [30].

CRY genes

CRY genes include *CRY1* and *CRY2*, in mice lacking *Cry1* and *Cry2*, the expression of melatonin is reduced, possibly because the pineal glands that control melatonin synthesis cannot perceive signals from the hypothalamus [31]. Barclay et al. [32] found that weight gain was significantly higher in $Cry^{-/-}$ mice than in wild-type mice under high-fat high-carbohydrate diets, indicating that mice with *Cry* mutations were more likely to develop diet-related obesity. In addition, *Cry1* also affects the male reproductive system [17, 33]; for example, in the testicular tissue of *Cry1*-knockout mice, testicular germ cell apoptosis is increased and sperm count is decreased [34].

PER genes

The *per* genes, first discovered in fruit flies, are also core genes in the circadian clock system. These genes have been detected in the human and mouse genomes, including *PER1*, *PER2*, and *PER3*. *Per* genes can act as tumor suppressors [35, 36]. In glioma tissue, the expression of *Per1* and *Per2* can promote apoptosis, a study has shown that *Per1* and *Per2* can increase the sensitivity of glioma cells to X-rays by promoting apoptosis mediated by the P53-dependent pathway [37]. Mutations or deletions in *Per2* accelerated the occurrence of liver cancer, suggesting that *Per2* is an inhibitor of liver cancer [38]. There is a close relationship between the *PERs* gene and the endocrine system. Estradiol can alter the expression patterns of *PER1* and *PER2* [39]. Reduced triiodothyronine levels (i.e., hypothyroidism) can entirely abolish the circadian rhythm mediated by the cardiac clock genes *Per2* and *Nr1d1* [40]. Further, thyroid hormones regulate the expression of *PER2* in the brain in a tissue-specific manner, in rats with thyroidectomy, the expression pattern of *PER2* in the elliptical nucleus of the stria bed nucleus and the amygdala nucleus was significantly altered, with no such effects in the SCN, basolateral amygdala, and dentate gyrus [41].

Effects of heavy metals on the circadian rhythm

The density of heavy metals is generally greater than 4.5 g/cm^3, including copper (Cu), Hydrargyrum (Hg), Cadmium (Cd), lead (Pb), chromium (Cr), and Manganese (Mn). A few heavy metal elements, such as Mn and Cu, are essential trace elements for the human body; however, all heavy metals exert toxic effects upon excessive accumulation in the body. The mechanisms underlying heavy metal toxicity have not been fully determined. Several recent studies have shown that heavy metals cause changes in circadian rhythm genes, which may contribute to the observed toxicity. Here, we discuss the effects of various heavy metals, including Cd, Mn, Cu, and Pb, on circadian rhythm genes.

Cd

Cd is a toxic heavy metal, and Cd discharged into the environment can enter water bodies or be absorbed by plants and animals. Cd enrichment in the human body can induce damage to the lungs, bones, brain, kidneys, and other important organs [42]. Cd can disturb the circadian rhythm in mammals by altering the expression of circadian rhythm genes. After one month of exposure of 45-day-old rats to low doses of $CdCl_2$, the circadian rhythm mediated by *Arntl* in the SCN was suppressed, the expression of *Clock* increased, the phases of *Per1/2* and *Cry2* were changed, and the normal rhythm of glutathione peroxidase (GPx) and glutathione reductase (GSR) was disturbed [43]. Correlations between the redox pathway and circadian rhythms have been demonstrated, and the glutathione system is important for maintaining the redox homeostasis of cells, which can remove peroxides *in vivo* [44]. There is a direct correlation between the redox state of nicotinamide adenine dinucleotide (NAD) and the expression of clock genes. The oxidative form of the redox cofactor NAD^+ inhibits the synthesis of CLOCK/ARNTL heterodimers, whereas the reduced form of NADH enhances the combination of the two [45]. In addition, the NAD^+-dependent deacetylase sirtuin3 (sirt3) is regulated by the circadian clock system, and its target, Mn superoxide dismutase (Mn-SOD), also exhibits 24 h rhythms [46]. As Cd affects the 24-h rhythmicity of GSR, GPx, and Mn-SOD, Cd may interfere with the rhythmicity of the circadian clock system via redox reactions (Figure 1). Melatonin treatment in rats exposed to $CdCl_2$ can eliminate the effects of $CdCl_2$ on *Per1* to some extent [47]. When rats were administered 5 ppm $CdCl_2$ in drinking water, the expression levels of *Per1* and *Per2* in the hypothalamus decreased, while melatonin treatment

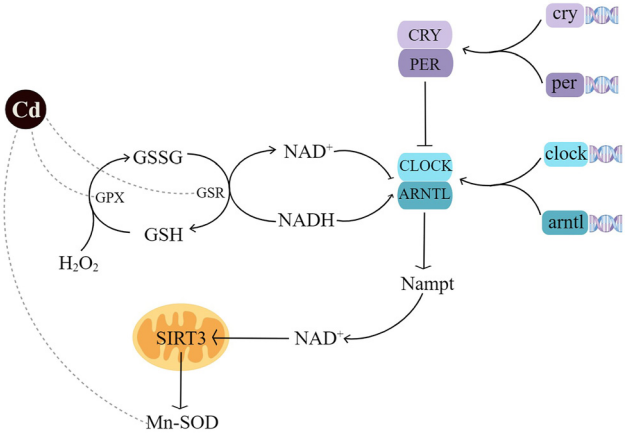

Figure 1: Relationships between the redox pathway and the circadian rhythm. Under the catalysis of GPx, GSH can neutralize H_2O_2, thereby converting it to GSSG in the oxidative state, and it can be reduced to GSH under the catalytic action of GSR. NAD^+ inhibits the binding of CLOCK and ARNTL, whereas NADH enhances the binding of CLOCK and ARNTL. NAD^+ regulated by the CLOCK/ARNTL heterodimer regulates the activity of SIRT3 in the mitochondria, and Mn-SOD is one of the targets of SIRT3. Cd disturbs the 24 h rhythmicity of GPx, GSR, and Mn-SOD. (Figures were created using Figdraw (www.figdraw.com)).

attenuated this adverse effect [48]. Xiao et al. [49] reared zebrafish larvae in tap water with 5 ppm $CdCl_2$ under 14 h light and 10 h dark and found that the mRNA levels of *clock1, per2,* and *arntl* changed significantly, suggesting that the mechanism by which Cd interferes with clock gene expression may involve the immune response and G protein-coupled receptor pathways. Cd is an endocrine disruptor [50]. The expression levels of *Arntl, Per*, and *Cry* in the pituitary gland as well as the circadian rhythms of prolactin and thyroid-stimulating hormone secreted by the pituitary gland are disturbed by Cd; however, melatonin administration can attenuate these changes to a certain extent [51].

Mn

Mn is an essential microelement and is of great significance for sustaining normal physiological functions. Mn mainly enters the human body through the respiratory and digestive systems, and high concentrations of Mn can damage the human nervous, respiratory, and reproductive systems. Few studies have evaluated the effects of Mn exposure on circadian rhythm genes. Bouabid et al. [52] used a rat model of Mn poisoning to explore the effects on circadian rhythm genes and found that the rest-activity rhythm of Mn-poisoned rats was significantly impaired. In rats exposed to Mn, the expression of *Arntl, Clock, Cry1,* and *Per* genes in the hypothalamus and liver was inhibited, leading to circadian rhythm disturbances [53].

Cu

Cu is also an essential trace element. In the normal adult human, Cu levels range from about 80–100 mg for the maintenance of normal physiological function. Both Cu excess and deficiency are associated with adverse effects [54]. Cu deficiency damages the cardiovascular, hematopoietic, and nervous systems, and Cu poisoning results in neurasthenia, nausea, vomiting, and liver and kidney failure [55]. Recent studies of the effects of Cu exposure on circadian rhythm genes have mainly focused on fish. Zebrafish exposed to ionic Cu showed alterations in circadian rhythm genes, especially *cry2* and *per2*, which were the first to be affected [56]. Doria et al. [57] examined the effects of copper exposure on circadian rhythm gene expression in zebrafish and found that Cu exposure interfered with the normal rhythmicity of *cry2*, *per1*, and *per2*, and the expression of catalase was associated with the expression of *cry1a* and *per2*. However, further research is necessary to explore the associations between antioxidant defense and circadian rhythm genes.

Pb

Pb is a potential carcinogen that enters the human body through the respiratory or digestive tract, resulting in neurotoxic effects, manifested as headaches, dizziness, memory loss, and sleep disturbance. In addition, excess Pb can cause damage to the hematopoietic system and kidneys [58]. In mice exposed to Pb for extended periods, the sleep-wake cycle is disturbed, the expression of *Per1* and *Per2* in the hypothalamus is increased, and the expression of *Arntl* is suppressed, suggesting that the disturbance caused by Pb in the sleep-wake cycle may be caused by the disruption of the expression of circadian rhythm genes in the hypothalamus [59]. The expression of clock genes is regulated by dopamine [60]. Sabbar et al. [61] reported that rats with Pb poisoning had lower levels of ARNTL, PER1, and PER2 but no changes in CRY1 and CRY2 levels. The authors also found that in rats with Pb poisoning, the concentration of dopamine in the striatum was reduced; therefore, circadian clock protein changes caused by Pb poisoning are likely related to dopamine levels.

Summary and outlook

Here, we summarized the effects of several common heavy metals on circadian rhythm genes (Table 1). Environmental compounds have been shown to affect circadian rhythms in animals. Dysregulation of the circadian rhythm affects many biological processes. As environmental pollutants, heavy metals can affect oxidative stress [62], endocrine functions [63], and the reproductive system [64]. Cd disrupts the activity of the hypothalamic-pituitary-gonadal axis [65], and it can disrupt circadian rhythm genes by acting as an endogenous interference, causing the rhythmic disruption of prolactin, thyrotropin, and luteinizing hormone [51]. The transcription of clock genes is sensitive to the redox state, and the daily rhythm of redox states in the hypothalamus requires the *Arntl* gene [66]. Exposure to heavy metals can also lead to the dysregulation of the redox state of cells [67]. These facts indicate that the mechanism underlying clock gene changes caused by heavy metals may be related to oxidative stress and the endocrine system.

In addition, we discussed various interesting phenomena that may help elucidate the mechanism of interactions between heavy metals and circadian genes. (1) The expression of rhythm genes may vary among tissues and organs. Under Cd exposure, *Per1* expression is reduced in the hypothalamus, with no significant expression change in the

Table 1: Effects of heavy metals on circadian rhythm genes.

Metal	Animal	Light cycle	Dosage and exposure time	Localization	Changes in circadian gene expression	Reference
Cd	Rats	12:12 h light/dark	5 ppm $CdCl_2$ in drinking water, 1 month	MBH (medial basal hypothalamus)	(1) *Arntl* was suppressed (2) *Per1* expression decreased at 17:00 and increased at 05:00	[43]
	Rats	12:12 h light/dark	5 ppm $CdCl_2$ in drinking water, 1 month	Pituitary	(1) *Clock* and *Arntl* were suppressed (2) The rhythmicity of *Per1* and *Cry2* was significantly disrupted	[51]
	Rats	12:12 h light/dark	5 ppm $CdCl_2$ in drinking water, 1 month	Hypothalamus Adenohypophy-sis	(1) Hypothalamus: *Per1* and *Per2* levels were reduced at 01:00 (2) Adenohypophysis: *Per1* and *Per2* levels were reduced at 09:00	[48]
	Zebrafish	14:10 h light/dark	5 ppm $CdCl_2$, 24 h	–	Levels of c*lock1*, *arntl, per2* were elevated during the light phase	[49]
Mn	Rats	12:12 h light/dark	1 mg/kg Mn^{2+}, 5 mg/kg Mn^{2+}	Hypothalamus Liver	(1) *Arntl* and *Clock* levels decreased in the hypothalamus and liver (2) Hypothalamus: *Per1* expression decreased (3) Liver: *Cry1* and *Per1/2* levels decreased	[53]
Cu	Zebrafish	–	10 µg Cu/L, 21 days	–	Levels of *cry2* and *per2* increased	[56]
Pb	Rats	12:12 h light/dark	250 ppm of lead acetate by drinking water, 5 weeks	Hypothalamus	*Per1* and *Per2* levels increased and *Arntl* levels decreased	[59]
	Rats	14:10 h light/dark	10 mg/kg lead acetate by intraperitoneal injection, 30 days	Hypothalamus	Levels of ARNTL, PER1, and PER2 were decreased	[61]

adenohypophysis [48]. This suggests that the mechanisms underlying cadmium-induced changes in circadian rhythm genes are highly complex and differ among organs. (2) Contradictory results have been reported. For example, Hsu et al. [59] found that *Per1* and *Per2* were upregulated when rats were kept under 12 h of light and were exposed to 250 ppm Pb acetate in drinking water. By contrast, in another study [61], rats exposed to 14 h light and treated with an intraperitoneal injection of 10 mg/kg Pb acetate showed decreased protein levels of PER1 and PER2. This discrepancy may be due to differences in heavy metal concentrations and modes of administration as well as differences in the duration of light; thus, further research is needed.

Environmental and industrial exposure to heavy metals is harmful to human health; however, the mechanisms underlying the toxic effects are not entirely clear and effective therapies are lacking. Further research on the effects of heavy metals on circadian rhythm genes may provide a basis for the development of novel therapeutic strategies for heavy metal poisoning.

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