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Effect of cisplatin on the clock genes expression in the liver, heart and kidney

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ABSTRACT

Cisplatin is a platinum-based chemotherapy drug that is widely used to treat various types of malignancies. Although the involvement of circadian clock in cisplatin metabolism and excretion has been reported, the effect of cisplatin on circadian rhythm remains unclear. In the present study, we investigated the effects of cisplatin on clock genes expression in mouse peripheral tissues. Cisplatin induced severe nephrotoxicity, as revealed by the significant increase of blood urea nitrogen and serum creatinine levels. Moreover, cisplatin circadian time-dependently induced *p21* expression in the liver, heart and kidney, with the highest increase during the dark phase. In addition, cisplatin altered the clock genes expression in the liver, heart and kidney in a tissue- and gene-specific manner. Interesting, the expression of D site of the albumin promoter binding protein (Dbp), a gene involved in detoxification and drug metabolism, was consistently suppressed in the liver, heart and kidney after cisplatin treatment, implying a role of DBP in the toxicity of cisplatin.

1. Introduction

Almost all organisms from bacteria to mammals possess a circadian clock that regulates physiological and behavioral processes to adapt to a 24 h diurnal cycle [1]. The mechanism of generating and maintaining the circadian oscillations in a single cell is the autoregulatory feedback loops of transcription and translation [2]. CLOCK and BMAL1 heterodimers bind to the E boxes in the promoters of *Cry* and *Per*. The accumulation of CRY and PER protein forms complexes that suppress the heterodimerization of BMAL1-Clock, resulting in the loss of activation of *Cry* and *Per* genes. And a reduction of CRY and PER protein levels leads to the next transcriptional cycling event. In addition, BMAL1-CLOCK heterodimers also drive the circadian expression of *Rev-erb*, which represses *Bmal1* and *Clock* gene transcription by binding to their promoters. This cell autonomous circadian oscillators exist in almost all mammalian cells and drives physiological oscillation by regulating the so called clock controlled genes.

The circadian clock is entrainable by light, food, drugs and toxicants [3,4]. The master circadian pacemaker in mammals, suprachiasmatic nucleus (SCN) is able to be entrained by light to adapt to the environment light-dark cycle. In addition, food as a cue is able to entrain the circadian clock in most peripheral tissues. Accumulating evidence indicate that circadian clock could also be altered by drugs and toxicants. For instance, carbon tetrachloride, which induces hepatic fibrosis in mice, leads to alterations in the circadian rhythms of hepatic clock genes [5]. Metformin, a popular anti-diabetic drug, affects the circadian clock in a tissue-specific manner [6]. Chronic ethanol-feeding also alters circadian clock genes expression in the livers of mice [7]. Further, ketamine alters the recruitment of the CLOCK:BMAL1 complex to the promoters of clock genes and clock-controlled genes in mouse embryonic fibroblasts [8].

Cisplatin is a platinum (Pt)-based chemotherapy drug that is widely used to treat various types of malignancies. Cisplatin also causes a series of side effects, including ototoxicity, gastrotoxicity, myelosuppression, neurotoxicity, allergic reactions and nephrotoxicity [9–11]. Renal manifestations after cisplatin treatment include acute kidney injury, hypomagnesemia, distal renal tubular acidosis, renal salt wasting, renal concentrating defect, hyperuricemia, transient proteinuria and chronic renal failure. The most serious side effect of cisplatin is acute kidney injury which occurs in 20–30% of patients [12,13].

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Previous studies have shown that clock-controlled genes, such as *Cyp2e1*, *Oct2* and *Mate1*, were involved in cisplatin metabolism and excretion [14,15]. In this study, we studied the effect of cisplatin in the clock genes expression in the liver, heart and kidney, in an attempt to gain new insights into the toxicity of this medicine.

2. Materials and methods

2.1. Animals

Mice were housed in groups of four to five per cage under controlled temperature (25 ± 1 °C) and a 12 h light/dark cycle (light on at 5 a.m., light off at 5 p.m.) with ad libitum access to food and water unless otherwise indicated. All animal procedures were performed under the ethical guidelines of Central South University.

2.2. Animal treatment

Mice were divided into 12 groups (n = 4–5) randomly. Six groups (n = 5 per group) of mice were intraperitoneally injected with a single dose of 20 mg/kg Cisplatin (Sigma Aldrich, St. Louis, MO, USA) at 7:00 (ZT2), 11:00 (ZT6), 15:00 (ZT10), 19:00 (ZT14), 23:00 (ZT18), and 02:00 (ZT22); control mice (n = 4 per group) received the equivalent volume of saline. Blood samples and tissues were collected 72 h after the injection. Serum was separated by centrifugation. Biochemical criterion were measured by routine methods used in the clinical laboratory of Xiangya hospital. Tissues were kept in −80 °C prior to analysis.

2.3. RNA isolation and RT-qPCR

Total RNA was extracted using Trizol reagent according to the manufacturer's instruction. 1 μg of total RNA were reverse-transcribed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Glen Burnie, MD, USA). The mRNA levels were examined with qPCR using SYBR Green PCR master mix (Fermentas, Glen Burnie, MD, USA) by C1000 touch Thermal Cycler (Bio-Rad, Hercules, CA, USA). Sequences for PCR primers are listed in Table S1. Relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$-method and normalized to the housekeeping gene *Gapdh*.

2.4. Data analysis

All data are given as the mean ± SEM. The values for the mRNA levels are presented as relative values in all experiments. One-way ANOVA was used to analyze circadian pattern with several time-points and to test the difference between more than two groups. Then the acrophase and amplitude of gene oscillation were calculated using the free Acrophase analysis software (Version 3.5 available at http://www.circadian.org/softwar.html). Two-way ANOVA was utilized to determine differences in body weight, biochemical criterion and gene expression between control and cisplatin-treated mice, followed by post hoc Bonferroni tests for pairwise comparison. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. The effects of cisplatin on mouse body weight and tissue functions

To titrate the doses for cisplatin treatment, we first analyzed renal function in C57/BL6 mouse at 72 h after intraperitoneal injection (i.p.) of different doses of cisplatin. As shown in Fig. 1A, blood urea nitrogen (BUN) and serum creatinine (Scr) levels were

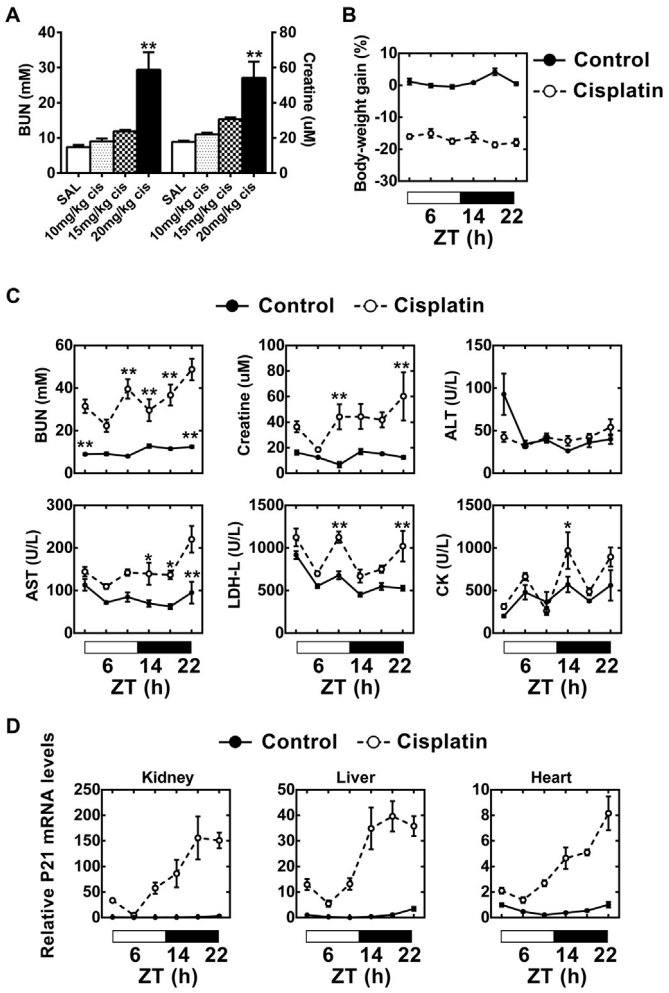

Fig. 1. Cisplatin affects body weight, tissue function and P21 mRNA expression. (A) 10, 15 or 20 mg/kg of cisplatin and equivalent volume of saline were intraperitoneally injected into mice, and blood samples were collected for measurements of BUN and Scr levels. (B, C, D) 20 mg/kg cisplatin and equivalent volume of saline were intraperitoneally injected into mice at ZT2, 6, 10, 14, 18 and 22, respectively. At 72 h after treatment, body weight were measured (B). Blood samples were collected for measurements of BUN, Scr, CK, ALT, AST and LDH-L levels (C). Total RNA was extracted and P21 mRNA expression levels were measured (D). Data were represented as the mean ± SEM of n = 4–5. * $p < 0.05$ and ** $p < 0.01$ compared with control group.

significantly increased after treated with 20 mg/kg cisplatin. This dose was used in the following experiments.

Next, 20 mg/kg cisplatin was intraperitoneal injected into mice at six different zeitgeber times (ZT; where ZT0 is the light on time, and ZT12 is the light off time). As shown in Fig. 1B, body weight was significantly reduced at 72 h after cisplatin treatment regardless of the injection time.

In addition, BUN and Scr levels were significantly elevated in the cisplatin-treated mice (Fig. 1C). The aspartate transaminase (AST) and L-lactate dehydrogenase (LDH-L) levels were also significantly increased in the cisplatin-treated mice, but the increase rate was lower than that of BUN and Scr (Fig. 1C). We did not find significant change in the alanine transaminase (ALT) level after cisplatin treatment and the creatine kinase (CK) level was only significantly increased at ZT14 (Fig. 1C).

Further, *p21* was significantly increased in the heart, liver and kidney after cisplatin treatment (Fig. 1D). The increase in *p21* expression showed a circadian rhythm in all tissues studies, with the highest increase at the night time and lowest increase during the day (One-way Anova, $P < 0.05$).