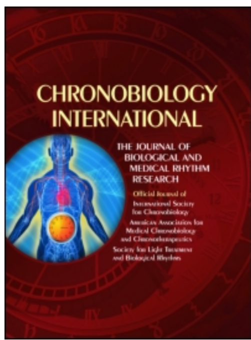

Taylor & Francis
Taylor & Francis Group

Chronobiology International
The Journal of Biological and Medical Rhythm Research

ISSN: 0742-0528 (Print) 1525-6073 (Online) Journal homepage: https://www.tandfonline.com/loi/icbi20

The Tilapia collagen peptide mixture TY001 protects against LPS-induced inflammation, disruption of glucose metabolism, and aberrant expression of circadian clock genes in mice

Xiao-Yun Xiong, Jun Liang, Yi-Qiao Xu & Yi Liu

To cite this article: Xiao-Yun Xiong, Jun Liang, Yi-Qiao Xu & Yi Liu (2019): The Tilapia collagen peptide mixture TY001 protects against LPS-induced inflammation, disruption of glucose metabolism, and aberrant expression of circadian clock genes in mice, Chronobiology International, DOI: 10.1080/07420528.2019.1606821

To link to this article: https://doi.org/10.1080/07420528.2019.1606821

Published online: 06 May 2019.

Submit your article to this journal

Article views: 44

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=icbi20

CHRONOBIOLOGY INTERNATIONAL
https://doi.org/10.1080/07420528.2019.1606821

Taylor & Francis
Taylor & Francis Group

REPORT

The Tilapia collagen peptide mixture TY001 protects against LPS-induced inflammation, disruption of glucose metabolism, and aberrant expression of circadian clock genes in mice

Xiao-Yun Xiong[a], Jun Liang[b], Yi-Qiao Xu[c], and Yi Liu[d]

[a]Yabao Institute of Health Sciences, Yabao Pharmaceutical Group Co., Ltd, Fenglingdu, Shanxi, China; [b]Yabao Production Center, Yabao Pharmaceutical Group Co., Ltd, Fenglingdu, Shanxi, China; [c]R&D department, Hunter Biotechnology, Inc, Hangzhou, Zhejiang, China; [d]The Center for Disease Control and Prevention of Shaanxi Province, Xi'an, Shaanxi, China

ABSTRACT

The Tilapia collagen peptide mixture TY001 has been shown to accelerate wound healing in streptozotocin-induced diabetic mice and to protect against streptozotocin-induced inflammation and elevation in blood glucose. The goals of the present study are to further study TY001 effects on lipopolysaccharide (LPS)-induced inflammation and metabolic syndrome. LPS is known to disrupt circadian clock to produce toxic effects, the effects of TY001 on rhythmic alterations of serum cytokines and hepatic clock gene expressions were examined. Mice were given TY001 (30 g/L, ≈ 40 g/kg) through the drinking water for 30 days, and on the 21st day of TY001 supplementation, LPS (0.25 mg/kg, ip, daily) was given for 9 days to establish the inflammation model. Repeated LPS injections produced inflammation, impaired glucose metabolism, and suppressed the expression of circadian clock core genes Bmal1 and Clock; clock feedback gene Cry1, Cry2, Per1, and Per2; clock target gene Rev-erbα and RORα. TY001 prevented LPS-induced elevations of TNFα, IL-1β, IL-6, and IL-10 in the liver, along with improved histopathology. TY001 reduced LPS-elevated fasting blood glucose and increased LPS-reduced serum insulin levels, probably via increased glucose transporter GLUT2, enhanced insulin signaling p-Akt and p-IRS-1^{Try612}. Importantly, LPS-induced circadian elevations of serum TNFα and IL-1β and aberrant expression of circadian clock genes in the liver were ameliorated by TY001. Immunohistochemistry revealed that the LPS decreased Bmal1 and Clock protein in the liver, which was recovered by TY001. Taken together, TY001 is effective against LPS-induced inflammation, disruption of glucose metabolism and disruption of circadian clock gene expressions.

Abbreviations: TY001: Tilapia collagen peptide mixture; LPS: Lipopolysaccharide; TNFα: Tumor necrosis factor-α; IL-1β: Interleukin-1β; GLUT2: Glucose transporter 2

ARTICLE HISTORY
Received 23 February 2019
Revised 20 March 2019
Accepted 9 April 2019

KEYWORDS
The collagen-peptide mixture TY001; LPS; inflammation; glucose metabolism; circadian rhythm; circadian clock gene expression

Introduction

The circadian clock is an endogenous biological timekeeping system located in every cell and organ to synchronize physiology and behavior to day/night cycles (Korencic et al. 2014). The pacemaker is located at the suprachiasmatic nucleus (SCN) in the anterior hypothalamus, while the regulator is composed of clock genes present in all peripheral tissues such as the liver (Adamovich et al. 2014; Mohawk et al. 2012). Once the physiological rhythms are disrupted, diseases occur (Froy 2012; Man et al. 2016; Maury et al. 2014).

Circadian clock genes are essential to drive and maintain circadian rhythm. The core clock genes include Bmal1, Clock, Npas2, the clock feedback control gene include Cry1, Cry2, Per1, and Per2, and the clock-controlled and/or clock-targeted genes include Rev-erbα, Dbp, Tef, etc. (Partch et al. 2014). Circadian clock genes have become the targets of toxicity and therapeutic applications (Dallmann et al. 2014; Li et al. 2017). Many natural products could affect the circadian clock to exert their beneficial effects (He et al. 2016; Li, 2017; Ribas-Latre et al. 2015).

The Tilapia collagen peptide mixture TY001 is a natural product. TY001 is the mixture of both animal and herbal peptides, aimed to compensate the shortage of essential amino acids from different protein resources (Xiong et al. 2018). TY001 is effective in promoting wound healing in acetic acid-induced

CONTACT Yi Liu sxjmkly@126.com The Center for Disease Control and Prevention of Shaanxi Province, Xi'an, Shaanxi, 710054, China
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/icbi.
supplemental data for this article can be accessed here

© 2019 Taylor & Francis Group, LLC

skin lesions in zebrafish (Xiong et al. 2018). TY001 is also effective in accelerating wound healing in streptozotocin (STZ)-induced diabetic mice, and in decreasing STZ-elevated blood glucose. Circadian rhythm regulates glucose metabolism (Pan and Hussain 2009; Reinke and Asher 2016; Shi et al. 2013; Stenvers et al. 2019) and effects of TY001 on glucose metabolism in relation to circadian rhythm is worth of investigation.

Circadian rhythms provide temporal regulation and coordination of immune responses to accelerate wound healing (Cable et al. 2017; Kowalska et al. 2013) and drives rhythmic fibroblast to promote skin wound healing (Hoyle et al. 2017). Circadian rhythm also regulates inflammatory response (Carter et al. 2016; Gachon et al. 2018). Circadian clock and inflammatory response are closely related (Man et al. 2016). The key component of circadian clock Bmal1 could regulate IL-1β production (Early et al. 2018); Cry1 could regulate the production of proinflammatory cytokines and regulate their release (Narasimamurthy et al. 2012). In *Clock-/-, Bmal1-/-, Cry1-/-, Cry2-/-, Per2-/-, Rev-erba-/-, and RORα-/-* mice, proinflammatory cytokines IL-1β, TNFα, and IL-6 increased, and other proinflammatory mediators Ccl2, Cxcl1, Cxcl5, NF-κB, and MIP2 also increased and/or altered (Carter et al. 2016). On the other hand, an inflammatory response could affect the circadian clock. For example, TNFα could affect E-box to suppress the expression of clock genes (Cavadini et al. 2007), and LPS suppressed clock gene expression in the brain (Li et al. 2018), ovary (Shimizu et al. 2017), and liver (Wang et al. 2016b), apparently via production of reactive oxygen species and inflammation. Using *TNFα-/-* mice, LPS-induced disruption of circadian rhythms and inflammation are found to be mediated by TNFα (Paladino et al. 2014)

The goal of the present study was to use multiple injections of LPS as a model to examine the effects of TY001 on LPS-induced inflammation, disturbance in glucose metabolism, insulin resistance, and importantly, on LPS-induced circadian disruption to provide the therapeutic basis of TY001 in inflammatory diseases, diabetes, and in promoting wound healing.

Materials and methods

Animals

Male C57 mice (25 ± 2 g) were obtained from the Fourth Military Medical University. Mice were housed in the animal facility according to the Animal Welfare of Chinese Guidelines, with free access to feed and the drinking water, maintained in a temperature of 22 ± 2°C, 12 h dark: 12 h light. Mice were acclimated for two weeks prior to experimentation. All the experimental procedures were approved by the Animal Ethics Committee of Forth Military Medical University.

LPS-induced disruption of circadian rhythm

LPS-induced diabetic model was established by multiple intraperitoneal injections of LPS (0.25 mg/kg in saline, ip), daily for consecutive 9 days. Twenty-four hr after the last LPS injection, blood and livers were collected at ZT1 (8:00), ZT7 (14:00), ZT13 (20:00), and ZT19 (2:00) for circadian rhythm analysis, and livers collected at ZT13 were used for histology and insulin signaling pathway analysis.

Experimental design

TY001 (patent granted by SIPO: 2017114143555) was composed of whey protein peptide concentrate, hydrolyzed wheat protein peptide, fish collagen peptide, calcium caseinate, wheat oligopeptide, and casein hydrolyzed peptide as described (Xiong et al. 2018). TY001 was dissolved in the drinking water as emulsions at the concentration of 30 g/L based on prior studies.

Mice were divided into three groups: LPS, LPS +TY001, and Control groups. The dose selection is based on the recommend clinical dose (20 g x 2 = 40 g for 60 kg/day). Taken the mouse/human convention faction of 9.1, and under the assumption that the body weight of mice was 25 g, and drunk 3–4 mL water/day, the dose of 30 g/L was equal to 37.92 –50.56 g/kg, and this dose was effective against STZ-induced inflammation and delay in wound healing in diabetic mice . Mice received TY001 for 21 days, followed by LPS challenge, and TY001 was continued to provide together with LPS for another 9 days, as illustrated in Figure 1. The duration of TY001

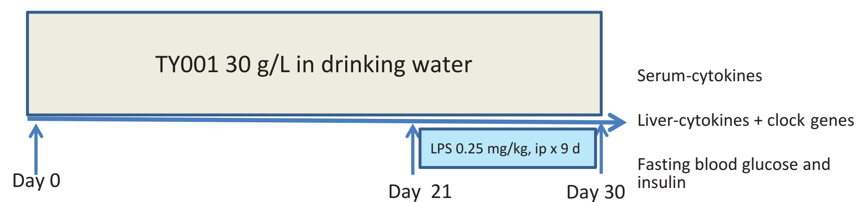

Figure 1. Experimental design.

treatment was based on a clinical trial (30 days), and our prior experience on wound healing in STZ diabetic mice.

After 30 days of TY001 supplementation and 9 days of LPS injection, Blood and livers were collected at ZT1, ZT7, ZT13 and ZT19 time point for biological analysis. At ZT13 (20:00), fasting blood glucose levels and fasting blood insulin levels were determined before collecting livers to examine the levels of inflammatory cytokines and insulin signaling pathway genes.

Histopathology and immunohistochemistry

Portion of the liver was fixed in 4% paraformaldehyde, embedded in paraffin, and cut 5 μm for standard H&E examination of pathological lesions under a light microscope.

For immunohistochemistry, sections were dewaxed in Xylene, and rehydrated in graded alcohols, and in 10 mM citrate buffer, microwaved for 10 min to expose antigen, followed by 3% H_2O_2 to block endogenous hydroperoxides. Slides were then incubated with primary antibodies against Bmal1 (Abcam, Cambridge, MA, 1:1000) and Clock (Abcam, 1:1000) at 4°C overnight, after washing with PBS, the second antibody (1:10000) was added. DAB was used to visualize the stain under a light microscope.

Western-blot analysis

Liver tissues were homogenized in RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing 1 mM phenylmethanesulfonylfluoride fluoride and freshly prepared proteinase inhibitors. Aliquoted proteins were denatured and separated on NUPAGE 10% BT gels and transferred to PVDF membranes. Membranes were blocked with 5% dry nonfat milk, followed by incubation with primary mouse antibody against β-actin, rabbit polyclonal antibodies against GLUT2, p-Akt, or p-IRS-1^{Tyr612} (1:1000) (Cell signaling Technology, Shanghai, China) overnight at 4°C. After washes with TBST, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit, anti-mouse IgG secondary antibodies (1:5000) for 1 h at room temperature. Protein–antibody complexes were visualized using an Enhanced Chemiluminescent reagent and a ChemiDoc XRS system (Bio Rad Laboratories, Inc., USA). Band intensities were semi-quantified by densitometry using Quantity One® software (version 4.6.2, Bio Rad Laboratories, Inc., USA)

Real-time RT-PCR

Portion of livers tissues (50 mg) were homogenized with Trizol (TaKaRa Biotechnology Co., Ltd., Dalian, China). The quality and quantity of total RNA were determined by measuring the absorbance at wavelengths of the 260/280 nm ratio. The total RNA was reverse transcribed to cDNA with the PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China). The IQ™ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) was used for real-time RT-PCR analysis. PCR reaction system is 20 μL contained 10 μL iQTM SYBR Green Supermix, 1.6 μL primer mix (10 μM each), 6.4 μL ddH2O and 2 μL cDNA (10 ng/μL). The thermocycling conditions were as follows: 5 min denaturation at 95°C; 40 cycles of annealing and extension at 60°C for 45 sec, and denaturation at 95°C for 15 sec. Dissociation curve was performed following the 40 cycles to verify the quality of primers and amplification. Primers were synthesized by AuGCT BioTach

(Beijing, China). Relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to the housekeeping gene β-actin, and the relative transcript levels were calculated as percentage of the housekeeping gene.

Serum cytokine determination

Serum IL-1β and TNFα levels were determined via commercial ELISA kits from Shanghai XinLe Biological (Shanghai, China), according to the instructions.

Statistics

All data were expressed as mean ± SEM. Comparison of multiple groups at each time point was performed using one-way ANOVA analysis followed by Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) software (version 16.0), and $p < .05$ was considered significantly different.

Results

TY001 protected against LPS-induced rhythmic elevations in serum cytokines

To examine LPS-induced rhythmic elevations of serum proinflammatory cytokines, blood was collected at ZT1 (8:00), ZT7 (14:00), ZT13 (20:00), and ZT19 (02:00), and serum proinflammatory IL-1β and TNFα were examined via ELISA. As shown in Figure 1, LPS markedly increased serum levels of IL-1β and TNFα, especially during the dark time (ZT13-ZT1), the time for animal to eat and drink. High levels of proinflammatory cytokine expression would affect mouse normal physiological functions. TY001 supplementation effectively ameliorated the elevations of these proinflammatory cytokines (Figure 2).

TY001 protected against LPS-induced liver injury and inflammation

Repeated LPS injection did not significantly affect animal body weight and liver weight (1.23 ± 0.12, 1.30 ± 0.20, and 1.32 ± 0.13 g for Control, LPS, and LPS + TY001, respectively), but produced mild liver injury as evidenced by inflammatory cell infiltration and spotted apoptosis (pink nuclei) (Figure 3). TY001 treatment significantly reduced inflammatory cell infiltration in the liver, and no pink cells were evident.

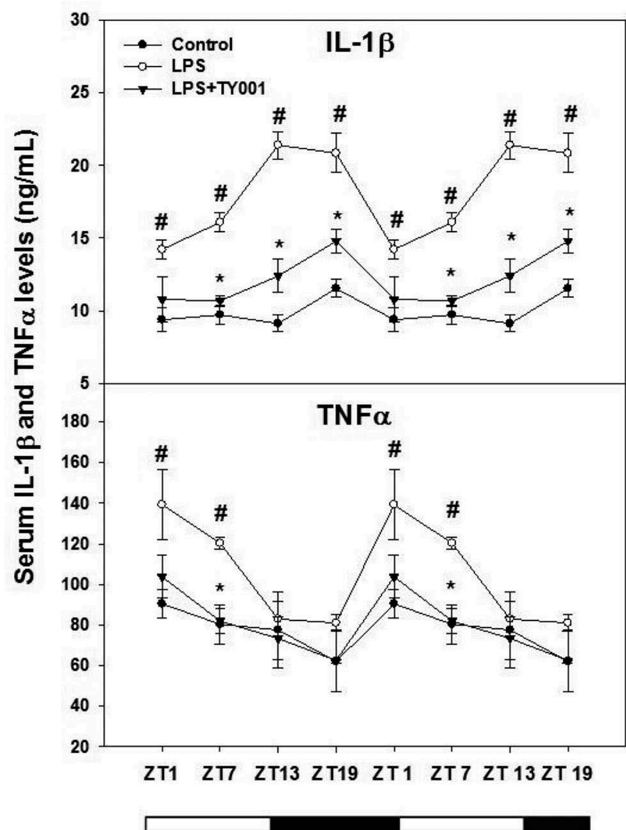

Figure 2. Effects of TY001 on LPS-induced rhythmic elevations serum IL-1β and TNFα levels. Mice were dosed with LPS (0.25 mg/kg, ip) daily for 9 days and blood was collected at ZT1, ZT7, ZT13, and ZT19, and serum cytokine levels were determined with ELISA kits. TY001 was administered through the drinking water for prevent phase (21 days), and treatment phase (9 days together with LPS), Saline only was used as controls. Data are mean + SEM of 6 mice.
#Significantly different from control, $p < .05$; *Significantly different from LPS, $p < .05$

To further confirm the inflammatory response in response to LPS injections, the expression of hepatic mRNA of major cytokines was determined via qPCR. As shown in Figure 3, LPS significantly increased the expression of TNFα, IL-1β, IL-6, and IL-10 in the liver, and TY001 supplementation decreased the overexpression of TNFα, IL-1β, and IL-10 (Figure 4). LPS also increased inflammatory protein expression of COX2 and iNOS in the liver, which was attenuated by TY001 (Supplementary Figure 1).

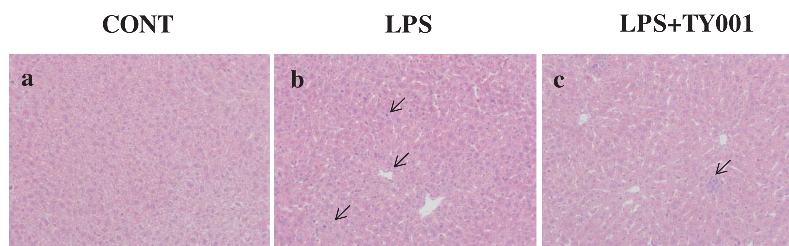

Figure 3. Representative photos of liver. Mice were dose with LPS (0.25 mg/kg, ip for 9 d), and liver histology showed inflammatory cell infiltration and spotted apoptosis (B) as compared to controls (A). C. TY001 supplementation (30 g/L in the drinking water) for 30 days (21 days pretreatment and continued 9 days together with LPS) ameliorated LPS-induced pathological lesions. Arrow indicates inflammatory cells. Magnitude (100x).

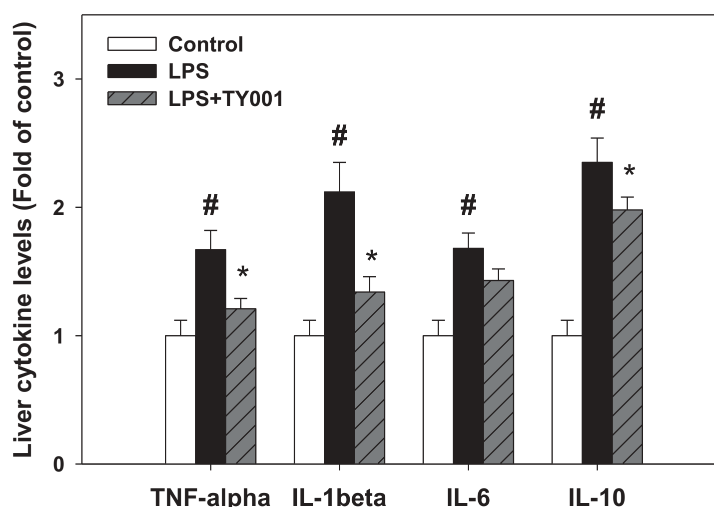

Figure 4. Effects of TY001 on TNFα, IL-1β, IL-6 and IL-10 levels in the liver. Mice were dose with LPS (0.25 mg/kg, ip) daily for 9 days and livers were collected 24 h after the last dose. TY001 was administered through the drinking water for prevent phase (21 days), and treatment phase (9 days together with LPS), Saline only was used as controls. Data are mean + SEM of 6 mice.
#Significantly different from control, $p < .05$; *Significantly different from LPS, $p < .05$

TY001 protected against LPS-induced disturbance in glucose metabolism

Fasting blood glucose levels and fasting blood insulin levels were determined at ZT13 (20:00) before collecting livers to examine the expression of insulin signaling pathway genes. As shown in Figure 5, repeated LPS injections significantly increased fasting blood glucose levels while decreased fasting insulin levels, indicating damage to the pancreas. TY001 supplementation ameliorated these adverse effects.

To further examine the signaling pathways by which TY001 improves insulin signaling, the GLUT2, p-Akt, and p-IRS-1 (Tyr612) were examined at the protein levels. Repeated LPS injections decreased the expression of glucose transport GLUT2 and glucose metabolism p-AKT, while increased insulin receptor substrate 1 (p-IRS-1) (Figure 6). TY001 promoted the phosphorylation of tyrosine, inhibited the downtrend of Akt phosphorylation, increased the expression of GLUT2, and reduced p-IRS-1 levels.

TY001 protected against LPS-induced suppression of circadian clock genes

In normal mouse livers, the oscillation of Clock, Bmal1, Cry1, Cry2, Per1, Per2, Rev-erbα, and RORα showed normal rhythm to ensure metabolism under physiological conditions. For example, the high expression of Bmal1 and Clock at the dark time (ZT13-ZT1) ensures the absorption of nutrient, metabolism and their use at the rest time. LPS reduced their expression peak values. The clock feedback gene Cry1, Cry2, Per1, and Per2 reached the

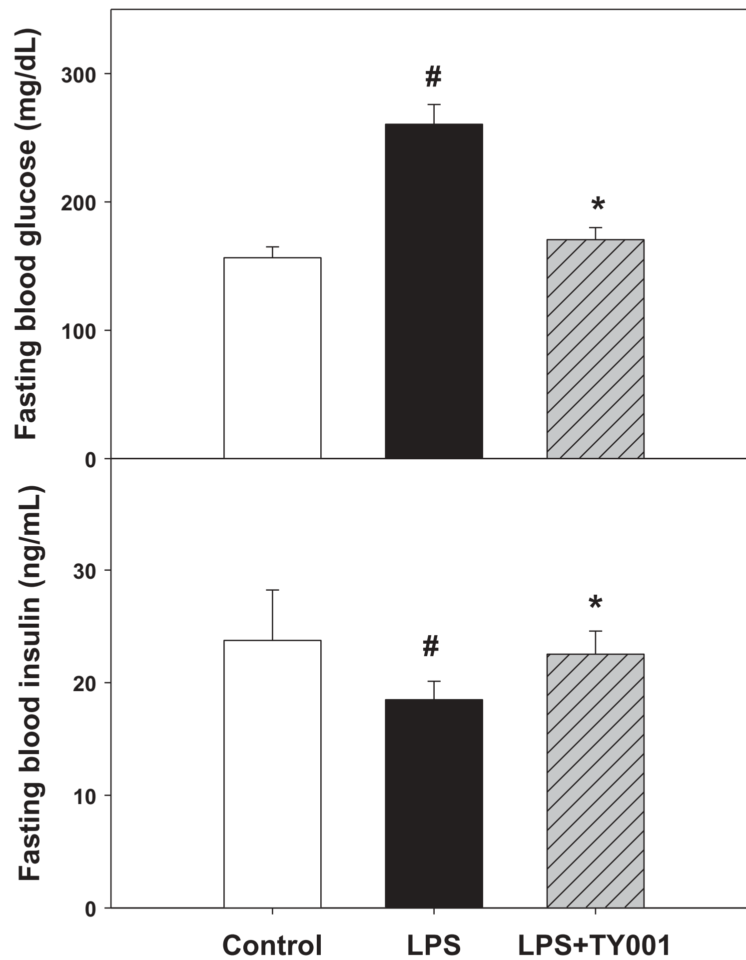

Figure 5. Effects of TY001 on fasting blood glucose and fasting serum insulin levels. Mice were dosed with LPS (0.25 mg/kg, ip) daily for 9 days and blood was collected 24 h after the last dose. TY001 was administered through the drinking water for prevent phase (21 days), and treatment phase (9 days together with LPS), Saline only was used as controls. Data are mean + SEM of 6 mice. #Significantly different from control, $p < .05$; *Significantly different from LPS, $p < .05$

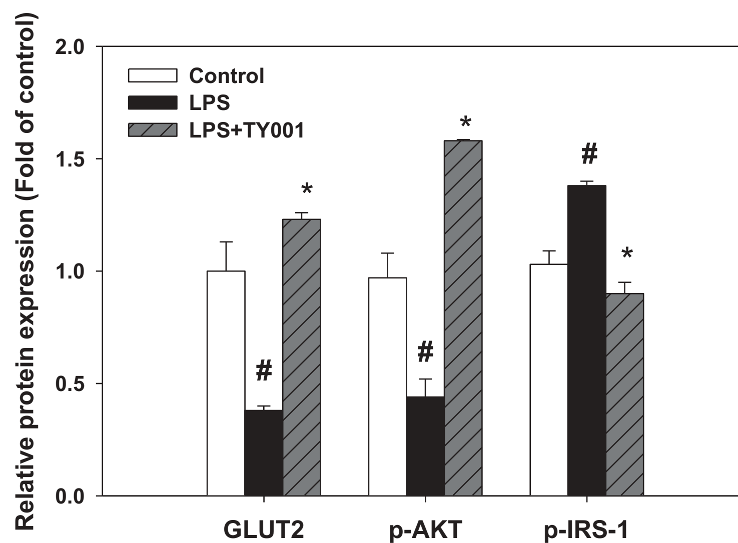

Figure 6. Effects of TY001 on glucose and insulin pathway gene expressions. Mice were dosed with LPS (0.25 mg/kg, ip) daily for 9 days and livers were collected 24 h after the last dose. TY001 was administered through the drinking water for prevent phase (21 days), and treatment phase (9 days together with LPS), Saline only was used as controls. Data are mean + SEM of 6 mice. #Significantly different from control, $p < .05$; *Significantly different from LPS, $p < .05$

peak at ZT13, LPS also reduced their peak expressions. LPS also caused the peak shift of Cry1 and RORα. All of these abnormal expressions could affect physiological metabolism. TY001 was effective to offset LPS effects on these aberrant expressions of clock genes, thus restoring normal metabolism rhythms (Figure 7).

The protein expression of the key clock proteins Bmal1, and Clock in the liver is shown in Figure 8. It is apparent that LPS decreased the intensity of these clock protein stains, which was reversed by TY001 treatments.

Discussion

The current study demonstrated that (1) multiple LPS injections produced rhythmic elevations of proinflammatory cytokines in the serum and caused inflammation, metabolic disorders, and disruption of circadian clock gene in the liver of

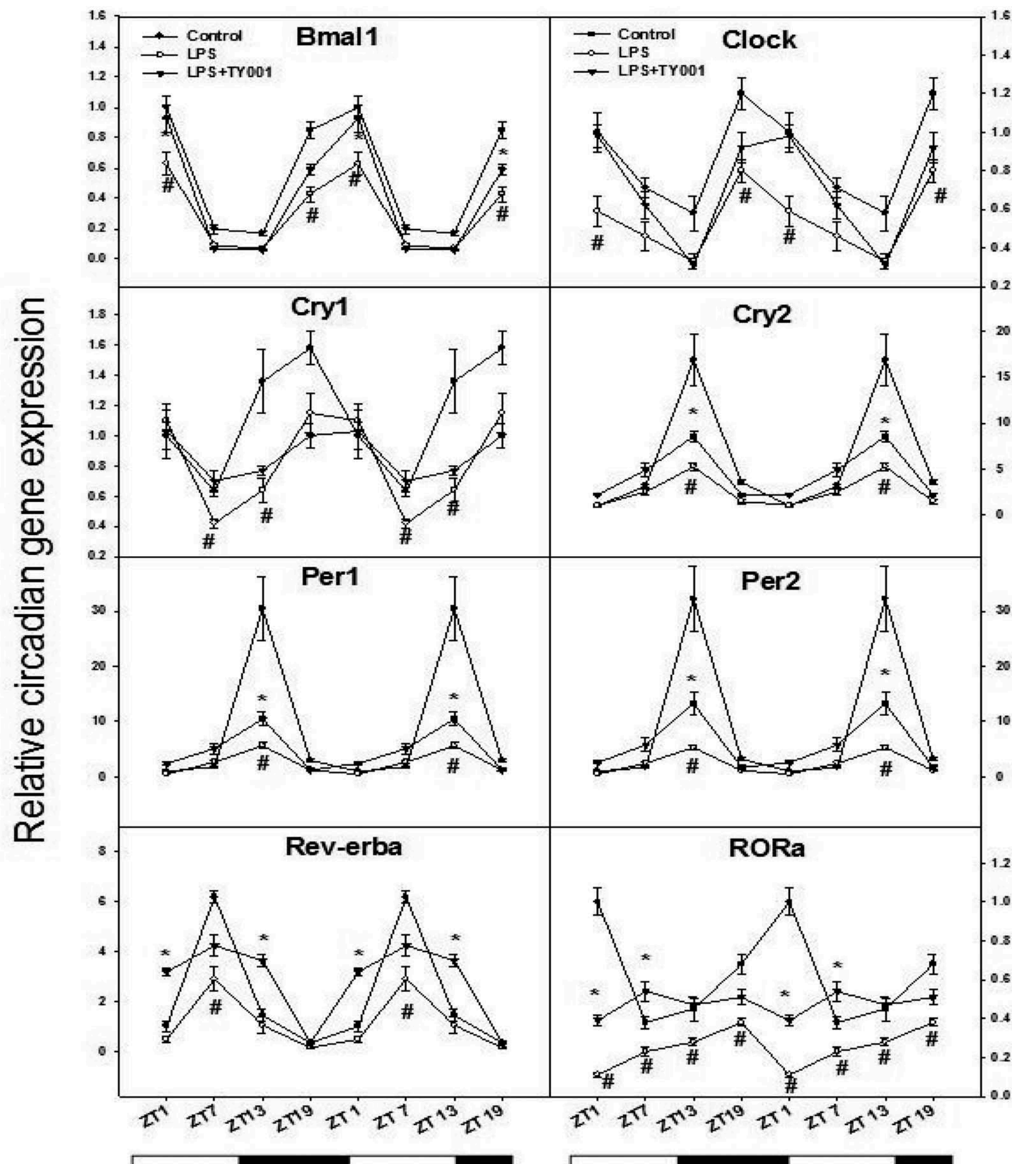

Figure 7. Effects of TY001 on hepatic circadian clock gene expression. Mice were dosed with LPS (0.25 mg/kg, ip) daily for 9 days and livers were collected at CT13 at (1 h after the last dose), ZT 7, ZT13, and ZT19, and serum cytokine levels were determined with real-time RT-PCR. TY001 was administered through the drinking water for prevent phase (21 days), and treatment phase (9 days together with LPS), Saline only was used as controls. Data are mean + SEM of 6 mice.

#Significantly different from control, $p < .05$; *Significantly different from LPS, $p < .05$

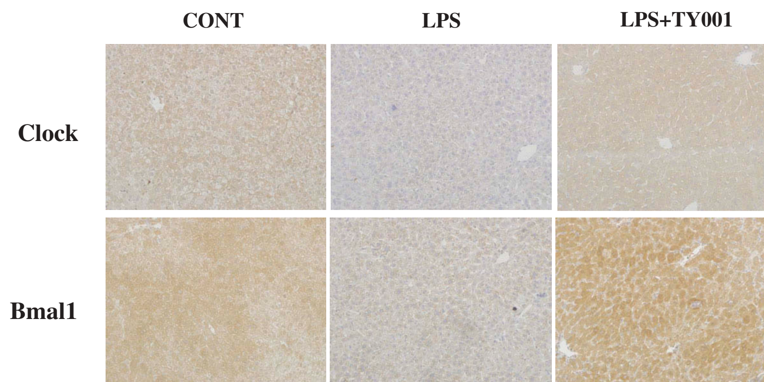

Figure 8. Representative figure of Bmal1 and Clock protein expression in the liver. Mice were dosed with LPS (0.25 mg/kg, ip) daily for 9 days and livers were collected at CT13 at 12 h after LPS were determined with real-time RT-PCR. TY001 was administered through the drinking water for prevent phase (21 days), and treatment phase (9 days together with LPS), Saline only was used as controls. Data are mean + SEM of 6 mice.

#Significantly different from control, $p < .05$; *Significantly different from LPS, $p < .05$.

C57BL/6 mice; (2) TY001 was effective in protecting against LPS-induced inflammation, elevations of fasting blood glucose, reduction of fasting serum insulin levels, probably mediated through increase glucose transporter GLUT2, insulin signaling pathway p-Akt, and p-IRS-1(Tyr612); and importantly (3) TY001 prevented LPS-induced rhythmic elevations of proinflammatory cytokines in the serum and reversed LPS-suppressed circadian clock gene expression in the liver.

Tilapia fish extracts as natural products have been shown to be beneficial in promoting wound healing (Chen et al. 2019; Hu et al. 2017; Ouyang et al. 2018). TY001 is the unique mixture of both animal and herbal peptides, composed of whey protein peptide concentrate, hydrolyzed wheat protein peptide, fish collagen peptide, calcium caseinate, wheat oligopeptide, casein hydrolyzed peptide, with high protein (80–90%) content (Xiong et al. 2018). TY001 is effective in protecting acetic acid-induced skin lesion in zebrafish (Xiong et al. 2018) and TY001 supplementation via the drinking water protected against STZ-induced inflammation, disturbance in glucose metabolism and the delay of diabetic wound healing. The novel findings of the current study uncovered that TY001 could also act on circadian clock to promote the absorption and metabolism of nutrients to maintain physiological homeostasis.

The core circadian molecular clock modulates the innate immune response by controlling rhythmic pathogen recognition through the innate immune system and daily variations in cytokine gene expression (Wang et al. 2016a). Repeated LPS-induced rhythmic elevation of proinflammation cytokines in the serum disrupted this normal rhythm. Disrupted circadian rhythms attenuated circadian clock gene expression in a ZT-specific manner to affect energy metabolism (Prendergast et al. 2015), including disrupted glucose metabolism in the present study, as evidenced by elevated fasting blood glucose, reduced fasting serum insulin levels, and altered glucose metabolism signaling pathways. TY001 significantly reduced fasting blood glucose and increased serum insulin levels. Mechanistically, TY001 increased glucose transporter GLUT2 to make more glucose uptake into the liver for metabolism and increased p-Akt and regulated p-IRS-1 to help glucose metabolism during diabetic and stress conditions (Liu et al. 2016; Rathinam and Pari 2016). More importantly, the beneficial effects of TY001 should also be attributed to maintain circadian clock gene expression (Figs. 6 and 7) to restore the dysregulated glucose metabolism (Stenvers et al. 2019; Yang et al. 2017).

Liver is a major target organ for LPS toxicity (Yang et al. 2018), as evidenced by histopathology and elevated pro-inflammatory cytokine TNF-α, IL-1β, and IL-6 at ZT13. Liver is also the target organ of LPS-induced chronotoxicity (Yamamura et al. 2010), as serum IL-1β was higher at all 4 ZT periods, particular from ZT13 to ZT19, while TNFα was higher at ZT1 to ZT7. Since circadian clock controls liver metabolic functions (Reinke and Asher 2016), and circadian disruption is the major cause of pathogenesis of

metabolic syndrome (Maury et al. 2014), including insulin resistance and elevated blood glucose levels (Bailey et al. 2014; Sadacca et al. 2011; Shi et al. 2013). Circadian clock dysregulation could further dysregulate immune function and inflammatory responses (Castanon-Cervantes et al. 2010; Gachon et al. 2018; Man et al. 2016). Thus, circadian clock is the potential target of chrono-pharmacology (Dallmann et al. 2014).

Liver is the critical site of peripheral circadian clock (Lamia et al. 2008). Overproduction of ROS by LPS damages the liver and disrupt the normal circadian rhythms (Wang et al. 2016b). TY001 supplementation not only protected LPS-induced toxicity to the liver but also restored the circadian clock to normal rhythm. As shown in Figure 6, LPS suppressed clock core gene Bmal1 and Clock at ZT19 and ZT1, which was prevented by TY001; LPS dramatically suppressed clock feedback gene Cry1, Cry2, Per1, and Per2, which was prevented or ameliorated by TY001; LPS also suppressed clock-controlled gene Rev-erba at ZT7 and RORα at all time points, which was ameliorated by TY001. In mammals, obesity and metabolic syndrome are closely related to circadian rhythms (Froy 2012; Gachon et al. 2018), and the clock is important for micronutrition absorption and utilization (Pan and Hussain 2009). Like many other natural products such as dietary proanthocyanidins (Ribas-Latre et al. 2015) Nobiletin (He et al. 2016), and many traditional medicines (Li HX and Liu 2017), TY001 effects on circadian clock function would help to correct LPS-induced inflammation and metabolic syndrome.

In summary, this study clearly showed that the Tilapia collagen-peptide mixture TY001 is effective in protecting against LPS-induced inflammation, the elevated fasting blood glucose via improved insulin signaling pathways. Importantly, TY001 restored LPS-suppressed circadian clock gene expression. TY001 is a promising candidate to restore normal circadian rhythmicity under pathological conditions.

Acknowledgements

The samples of TY001 are provided by Yabao Pharmaceutical Group Co, Ltd. (Shanxi, China).

Funding

This work was supported by the National Key Research and Development Program of China [2016YFD0400602].

Author Contributions

X-Y.X., and Y.L. conceived and designed the experiments; X-Y. X., J.L, and Y.X. performed the experiments; X-Y.X., J.L. and Y. L. analyzed the data; X-Y.X. and Y.L. wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

Adamovich Y, Rousso-Noori L, Zwighaft Z, Neufeld-Cohen A, Golik M, Kraut-Cohen J, Wang M, Han X, Asher G. 2014. Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. Cell Metab. 19:319–330.

Bailey SM, Udoh US, Young ME. 2014. Circadian regulation of metabolism. J Endocrinol. 222:R75–96.

Cable EJ, Onishi KG, Prendergast BJ. 2017. Circadian rhythms accelerate wound healing in female Siberian hamsters. Physiol Behav. 171:165–174.

Carter SJ, Durrington HJ, Gibbs JE, Blaikley J, Loudon AS, Ray DW, Sabroe I. 2016. A matter of time: Study of circadian clocks and their role in inflammation. J Leukoc Biol. 99:549–560.

Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, Besing RC, Menaker M, Gewirtz AT, Davidson AJ. 2010. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol (Baltimore, Md: 1950). 185:5796–5805.

Cavadini G, Petrzilka S, Kohler P, Jud C, Tobler I, Birchler T, Fontana A. 2007. TNF-alpha suppresses the expression of clock genes by interfering with E-box-mediated transcription. Proc Natl Acad Sci U S A. 104:12843–12848.

Chen J, Gao K, Liu S, Wang S, Elango J, Bao B, Dong J, Liu N, Wu W. 2019. Fish collagen surgical compress repairing characteristics on wound healing process In Vivo. Mar Drugs. 17: 1–12. doi:10.3390/md17010033

Dallmann R, Brown SA, Gachon F. 2014. Chronopharmacology: New insights and therapeutic implications. Annu Rev Pharmacol Toxicol. 54:339–361.

Early JO, Menon D, Wyse CA, Cervantes-Silva MP, Zaslona Z, Carroll RG, Palsson-McDermott EM, Angiari S, Ryan DG, Corcoran SE, et al. 2018. Circadian clock protein BMAL1 regulates IL-1beta in macrophages via NRF2. Proc Natl Acad Sci U S A. 115:E8460–e8468.

Froy O. 2012. Circadian rhythms and obesity in mammals. ISRN Obesity. 2012:437198.

Gachon F, Yeung J, Naef F. 2018. Cross-regulatory circuits linking inflammation, high-fat diet, and the circadian clock. Genes Dev. 32:1359–1360.

He B, Nohara K, Park N, Park YS, Guillory B, Zhao Z, Garcia JM, Koike N, Lee CC, Takahashi JS, et al. 2016. The small molecule nobiletin targets the molecular oscillator to enhance circadian rhythms and protect against metabolic syndrome. Cell Metab. 23:610–621.

Hoyle NP, Seinkmane E, Putker M, Feeney KA, Krogager TP, Chesham JE, Bray LK, Thomas JM, Dunn K, Blaikley J, et al. 2017. Circadian actin dynamics drive rhythmic fibroblast mobilization during wound healing. Sci Transl Med. 9: 1–10.doi: 10.1126/scitranslmed.aal2774

Hu Z, Yang P, Zhou C, Li S, Hong P. 2017. Marine collagen peptides from the skin of Nile Tilapia (Oreochromis niloticus): Characterization and wound healing evaluation. Mar Drugs. 15: 1–11.doi:10.3390/md15040102

Korencic A, Kosir R, Bordyugov G, Lehmann R, Rozman D, Herzel H. 2014. Timing of circadian genes in mammalian tissues. Sci Rep. 4:5782.

Kowalska E, Ripperger JA, Hoegger DC, Bruegger P, Buch T, Birchler T, Mueller A, Albrecht U, Contaldo C, Brown SA. 2013. NONO couples the circadian clock to the cell cycle. Proc Natl Acad Sci U S A. 110:1592–1599.

Lamia KA, Storch KF, Weitz CJ. 2008. Physiological significance of a peripheral tissue circadian clock. Proc Natl Acad Sci U S A. 105:15172–15177.

Li H, Fan X, Luo Y, Song S, Liu J, Fan Q. 2017. Repeated manganese administration produced abnormal expression of circadian clock genes in the hypothalamus and liver of rats. Neurotoxicology. 62:39–45.

Li H, Song S, Wang Y, Huang C, Zhang F, Liu J, Hong JS. 2018. Low-grade inflammation aggravates rotenone neurotoxicity and disrupts circadian clock gene expression in rats. Neurotox Res. doi:10.1007/s12640-018-9982-3

Li HX SF, Liu J. 2017. Circadian clock gene expression is the novel therapeutic targets of traditional medicines. Zhouhua Zhongyiyao Zazhi. 34:5464–5467.

Liu B, Yu CJ, Meng XB, Wen FC, Zhao HW, Sun GB, Ding T, Xu HB, Sun XB. 2016. [Effects of qizhi jiangtang capsule on protein expressions of InsR, PI3K, GLUT2 and p-JNK in hepatic tissues of rats with type 2 diabetes]. Zhongguo Zhong Yao Za Zhi. 41:1978–1982.

Man K, Loudon A, Chawla A. 2016. Immunity around the clock. Science (New York, NY). 354:999–1003.

Maury E, Hong HK, Bass J. 2014. Circadian disruption in the pathogenesis of metabolic syndrome. Diabetes Metab. 40:338–346.

Mohawk JA, Green CB, Takahashi JS. 2012. Central and peripheral circadian clocks in mammals. Annu Rev Neurosci. 35:445–462.

Narasimamurthy R, Hatori M, Nayak SK, Liu F, Panda S, Verma IM. 2012. Circadian clock protein cryptochrome regulates the expression of proinflammatory cytokines. Proc Natl Acad Sci U S A. 109:12662–12667.

Ouyang QQ, Hu Z, Lin ZP, Quan WY, Deng YF, Li SD, Li PW, Chen Y. 2018. Chitosan hydrogel in combination with marine peptides from tilapia for burns healing. Int J Biol Macromol. 112:1191–1198.

Paladino N, Mul Fedele ML, Duhart JM, Marpegan L, Golombek DA. 2014. Modulation of mammalian circadian rhythms by tumor necrosis factor-alpha. Chronobiol Int. 31:668–679.

Pan X, Hussain MM. 2009. Clock is important for food and circadian regulation of macronutrient absorption in mice. J Lipid Res. 50:1800–1813.

Partch CL, Green CB, Takahashi JS. 2014. Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 24:90–99.

Prendergast BJ, Cable EJ, Stevenson TJ, Onishi KG, Zucker I, Kay LM. 2015. Circadian disruption alters the effects of lipopolysaccharide treatment on circadian and ultradian locomotor activity and body temperature rhythms of female Siberian Hamsters. J Biol Rhythms. 30:543–556.

Rathinam A, Pari L. 2016. Myrtenal ameliorates hyperglycemia by enhancing GLUT2 through Akt in the skeletal muscle and liver of diabetic rats. Chem Biol Interact. 256:161–166.

Reinke H, Asher G. 2016. Circadian clock control of liver metabolic functions. Gastroenterology. 150:574–580.

Ribas-Latre A, Baselga-Escudero L, Casanova E, Arola-Arnal A, Salvado MJ, Blade C, Arola L. 2015. Dietary proanthocyanidins modulate BMAL1 acetylation, Nampt expression and NAD levels in rat liver. Sci Rep. 5:10954.

Sadacca LA, Lamia KA, deLemos AS, Blum B, Weitz CJ. 2011. An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. Diabetologia. 54:120–124.

Shi SQ, Ansari TS, McGuinness OP, Wasserman DH, Johnson CH. 2013. Circadian disruption leads to insulin resistance and obesity. Current Biology: CB. 23:372–381.

Shimizu T, Watanabe K, Anayama N, Miyazaki K. 2017. Effect of lipopolysaccharide on circadian clock genes Per2 and Bmal1 in mouse ovary. J Physiol Sci. 67:623–628.

Stenvers DJ, Scheer F, Schrauwen P, la Fleur SE, Kalsbeek A. 2019. Circadian clocks and insulin resistance. Nat Rev Endocrinol. 15:75–89.

Wang T, Wang Z, Yang P, Xia L, Zhou M, Wang S, Du J, Zhang J. 2016a. PER1 prevents excessive innate immune response during endotoxin-induced liver injury through regulation of macrophage recruitment in mice. Cell Death Dis. 7:e2176.

Wang Y, Pati P, Xu Y, Chen F, Stepp DW, Huo Y, Rudic RD, Fulton DJ. 2016b. Endotoxin Disrupts Circadian Rhythms in Macrophages via Reactive Oxygen Species. PLoS One. 11:e0155075.

Xiong XY, Liu Y, Shan LT, Xu YQ, Liang J, Lai YH, Hsiao CD. 2018. Evaluation of collagen mixture on promoting skin wound healing in zebrafish caused by acetic acid administration. Biochem Biophys Res Commun. 505:516–522.

Yamamura Y, Yano I, Kudo T, Shibata S. 2010. Time-dependent inhibitory effect of lipopolysaccharide injection on Per1 and Per2 gene expression in the mouse heart and liver. Chronobiol Int. 27:213–232.

Yang YQ, Yan XT, Wang K, Tian RM, Lu ZY, Wu LL, Xu HT, Wu YS, Liu XS, Mao W, et al. 2018. Triptriolide Alleviates Lipopolysaccharide-Induced Liver Injury by Nrf2 and NF-kappa B signaling pathways. Front Pharmacol. 9:999.

Yang Z, Kim H, Ali A, Zheng Z, Zhang K. 2017. Interaction between stress responses and circadian metabolism in metabolic disease. Liver Research. 1:156–162.