MEGA JOURNAL OF CASE REPORTS

OPEN ACCESS

Research Article

Compiled Date: February 28, 2024

Chronic Exposure to Subtherapeutic Levels of Benzylpenicilloic Acid Mediates Hepatorenal Dysfunction in Murine Models

Ruixue Hu[#], Jian Guo[#], Ke Zhao, Honglin Ren, Zengshan Liu, Shiying Lu, Yansong Li, Yang Wang[*] and Pan Hu[*]

State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, Jilin 130062, China

[*]**Corresponding author**: Pan Hu, State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, Jilin 130062, China, E-mail: hupan84@163.com

Yang Wang, State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, Jilin 130062, China, E-mail: ccwangy@126.com

[#]These authors contributed equally to this work.

Abstract

Benzylpenicilloic acid (BPNLA) is a byproduct of the natural degradation and enzymatic hydrolysis of penicillin. BPNLA primarily enters and accumulates in the human body through the consumption of animal products. Previous research has mainly focused on drug resistance and the resulting allergic reactions, but as the accumulation of the drug increases, toxicity also manifests. The liver and kidneys are the main organs for drug metabolism and excretion of many drugs and are most prone to toxic effects. Therefore, this study aimed to investigate whether BPNLA causes hepatorenal toxicity. All C57BL/6 mice were randomly assigned to four groups and administered orally and topically 2.925, 146.25, and 7312.5 μg/kg b.wt (body weight) of BPNLA or an equivalent volume of vehicle (control) for 35 days. The results showed that BPNLA could lead to a decrease in the organ coefficient of the liver, as well as structural abnormalities in the liver and kidneys. Further research found that liver and kidney function markers, lipid peroxidation markers (MDA), and proinflammatory cytokines (TNF-α and IL-1β) compared to the control group significantly increased. Moreover, the levels of antioxidant markers (GSH, SOD, GPX) decreased and showed a dose-dependent manner. In summary, the

results clearly demonstrated that even relatively low concentrations of BPNLA can cause liver and kidney damage, highlighting the need for concern regarding human exposure to BPNLA.

Keywords: Benzylpenicilloic acid; Hepatorenal toxicity; Lipid peroxidation; Oxidative stress; Inflammation

Introduction

Antibiotics, particularly β-lactam antibiotics, are extensively used in agriculture due to their strong antibacterial properties, low toxicity, and affordability [1]. Among the β-lactam antibiotics, Penicillin G has garnered significant attention in the livestock industry [2]. However, due to the widespread use of penicillin, drug resistance has emerged, and several countries have proposed measures to restrict its use [3]. In addition to the emergence of drug resistance, there are also cases of liver injury after the use of penicillin in clinical practice [4]. In addition, penicillin has also been detected in urine [5], suggesting that penicillin may have potential hepatotoxic and nephrotoxic effects. The liver and kidney are the primary organs responsible for metabolizing Penicillin G. It is mainly excreted through the kidneys, with renal tubular secretion being the primary mechanism in adults [6]. Research on the half-life of penicillin has indicated that the terminal half-life of penicillin G is 3.5 hours in the kidney and 3.0 hours in the liver [7]. The terminal half-life in both the liver and kidney is similar. However, higher concentrations and greater drug residues are found in the liver. A study demonstrated that shortly after consuming a milk substitute containing penicillin, the concentration of penicillin in the liver tissue of calves exceeded the maximum allowable residue concentration [7], indicating that the liver tissue contains the highest concentration of penicillin G. After being digested and absorbed into the liver, Penicillin G is degraded into various by-products, with Benzylpenicilloic Acid (BPNLA) being the main degradation product [8]. Additionally, BPNLA has also been detected in urine [9], indicating that penicillin G can also be degraded in the kidney. These results suggested that BPNLA can accumulate in liver and kidney tissue, potentially posing harm to human health. Previous studies have shown that BPNLA exhibits cytotoxic [10], although its toxicity towards liver and kidney cells is relatively low and warrants further investigation. The hepatorenal toxicity resulting from long-term exposure to BPNLA has not been adequately studied.

Multiple studies have demonstrated a connection between oxidative stress and liver and kidney damage development, including inflammation, hypertrophy, and fibrosis, all marked by disturbances in redox signaling and control [11-14]. However, it remained uncertain whether BPNLA caused hepatotoxicity and nephrotoxicity through the mechanism of oxidative stress. In this study, we established a gavage-feeding model in mice to evaluate the effects of different doses of BPNLA on bioaccumulation and liver and kidney function injury by measuring organ coefficients, biochemical indicators of liver and kidney function, oxidative stress markers, and inflammatory cytokine levels, as well as observing histopathology. This study aims to provide valuable insights for the safety assessment of antibiotic residues.

Material and Methods

Animals

Male C57BL/6 mice (18-22g) were purchased from the Experimental Animal Center of Jilin University. The mice were housed in standard animal cages with controlled environmental temperature and humidity for one week to acclimatize. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Jilin University. All procedures follow the National Institutes

of Health guidelines for the care and use of laboratory animals.

Experimental design

The mice were randomly divided into four groups (n=4), including the control group, the low dose group (2.925 μg/kg/day), the medium dose group (146.25 μg/kg/day), and the high dose group (7312.5 μg/kg/day) BPNLA. BPNLA (purity≥97%, Wang zhixing, China) was dissolved in Dimethyl Sulfoxide (DMSO) to prepare a stock solution. This stock solution was then diluted to the desired concentration with 0.9% saline before use. The lowest dose of BPNLA was determined based on the acceptable daily intake for humans in combination with the conversion factor between humans and mice. The control group received an equal volume of normal saline and was administered via gavage once a day for 35 consecutive days. After the experiment, the mice were sacrificed and blood, as well as liver/kidney tissue, were collected for further analysis.

Assessment of liver and kidney organ coefficients

After a 24-hour fasting period without water deprivation, the body weight of each mouse was measured before they were euthanized. The liver and kidney tissues were removed and weighed, then the organ coefficients were recorded and calculated. The organ coefficient calculation method is as follows: Organ coefficient = organ weight/body weight × 100%.

ELISA assay

According to the manufacturer's instructions, the levels of TNF-α and IL-1β in liver and kidney tissues were determined using corresponding ELISA kits (Biolegend, USA).

Quantitative Real-Time PCR assay

According to the manufacturer's instructions, total RNA was extracted from liver and kidney tissues, and the samples were reverse transcribed into cDNA using a kit. Quantitative evaluation of cDNA amplification for each gene was performed using a fluorescence-based real-time detection method using the fluorescent SYBR Green dye (Thermo Scientific, USA). The primer sequences used for RT-PCR analysis are shown in **Table 1**. Each qRT-PCR was performed with three biological replicates, and each biological replicate was evaluated three times. The $2^{-\Delta\Delta CT}$ method was used to calculate and analyze the comparative threshold cycle (Ct) values.

Table 1: Primer sequences.

Gene	Sequences (5'-3')	Length (bp)	Accession No
TNF-α	F: CCTATGTCTCAGCCTCTTCTCAT		
	R: CACTTGGTGGTTTGCTACGA	214	NM_008361.4
IL-1β	F: ACCTGTGTCTTTCCCGTGG		
	R: TCATCTCGGAGCCTGTAGTG	162	NM_008361.4
GAPDH	F: AGGTCGGTGTGAACGGATTTG	95	NM_001289726.2

Determination of liver and kidney function markers

According to the manufacturer's instructions, the levels of Alanine Aminotransferase (ALT), Aspartate

Aminotransferase (AST), Blood Urea Nitrogen (BUN), High-Density Lipoprotein Cholesterol (HDL-c), Total Bile Acid (TBA) and Triglycerides (TG) in liver and kidney tissues were determined using commercially available detection kits (Nanjing Jiancheng Institute of Biotechnology, China) as a sensitive indicator of liver and kidney injury.

Analysis of oxidative stress markers

The supernatant obtained from the grinding and centrifugation of liver and kidney tissues was used for the analysis of oxidative indicators. The levels of Superoxide Dismutase (SOD) (Beyotime, China), Glutathione (GSH) (Solarbio, China), Glutathione Peroxidase (GPX) (Solarbio, China), and Malondialdehyde (MDA) (Solarbio, China) were determined using commercial detection kits.

Histopathological examination

Part of the liver and kidney tissues were fixed in 4% paraformaldehyde, dehydrated in graded ethanol, and embedded in paraffin. The tissue blocks were cut into 4 μm sections, stained with hematoxylin and eosin, and histologically examined using a light microscope (Olympus, Japan).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0 (San Diego, USA). Data for each group were presented as mean ± Standard Error (SE). One-way Analysis of Variance (ANOVA) was used for statistical analysis, followed by Tukey's test to determine differences between groups. A value of $p < 0.05$ was considered statistically significant.

Results

The effect of BPNLA on body weight and organ coefficients of liver and kidney

Sixteen male C57BL/6 mice were randomly divided into four groups to evaluate the *in vivo* toxicity of BPNLA. All animals survived during the experiment and were euthanized at the end of the experiment. The body weight of each mouse was recorded at the beginning and end of the experiment. All data are presented in **Table 2**. The results showed that, compared with the control group, although there was a certain dose-dependent relationship between the dose of BPNLA and the body weight of mice, the feeding of BPNLA at all doses did not cause significant weight loss in mice. However, unexpectedly, we found that although low-dose BPNLA treatment had little effect on the liver, the liver organ coefficient of mice in the high-dose group significantly decreased ($p < 0.05$). Since BPNLA is an important metabolite of penicillin, this suggested that the extensive use of penicillin not only causes known drug resistance, but also may lead to potential liver damage through the accumulation of BPNLA.

Table 2: Effect of BPNLA on male mice body and organs weights.

	Control	2.925 μg/kg/day	146.25 μg/kg/day	7312.5 μg/kg/day
Weight change (g)	6.823±1.398	6.745±2.346	9.133±4.449	7.793±3.073
Liver weight (g)	1.25±0.059	1.247±0.142	1.029±0.119	0.9681±0.196
Kidney weight (g)	0.3288±0.0278	0.3222±0.0743	0.3187±0.03857	0.3041±0.0206
Liver/wt ratio	0.05079±0.0006	0.05019±0.0028	0.04145±0.0042*	0.04343±0.0008*
Kidney/wt ratio	0.01315±0.0001	0.01210±0.0005	0.01283±0.0013	0.01259±0.0006

Results are expressed as the mean ±SEM. Different superscripts within the same column designate significant differences ($p < 0.05$).

Pathological damage of liver and kidney induced by BPNLA

Histological analysis showed that the hepatocytes in the liver tissue of the control group were arranged in a regular pattern with normal liver parenchyma, hepatic cords central veins, and sinusoids. However, all experimental groups showed enlarged interstitial spaces, significant hemorrhage, and infiltration with inflammatory cells in the liver tissue, with the extent of infiltration increasing with increasing doses. In the low-dose BPNLA group, the interstitial spaces of the liver were slightly enlarged and accompanied by slight bleeding. In the medium-dose group, the hepatic sinusoids and hepatic cords were significantly affected. In the highest dose group, there was severe bleeding and extensive vacuolization of the hepatocytes **(Figure 1A-D)**. In the control group, the renal glomeruli and renal tubular epithelium were densely packed without significant inflammation. In the low-dose group, glomerular hemorrhages and swelling of the renal tubular epithelium were observed. Renal tissue lesions were evident in the medium and high-dose groups. The lesions included focal necrosis of the renal tubular epithelium, nuclear pyknosis, and inflammatory cell infiltration, and these changes were dose-dependent **(Figure 1E-H)**. Although BPNLA did not cause a decrease in kidney index, pathological testing proved that it could cause liver and kidney damage.

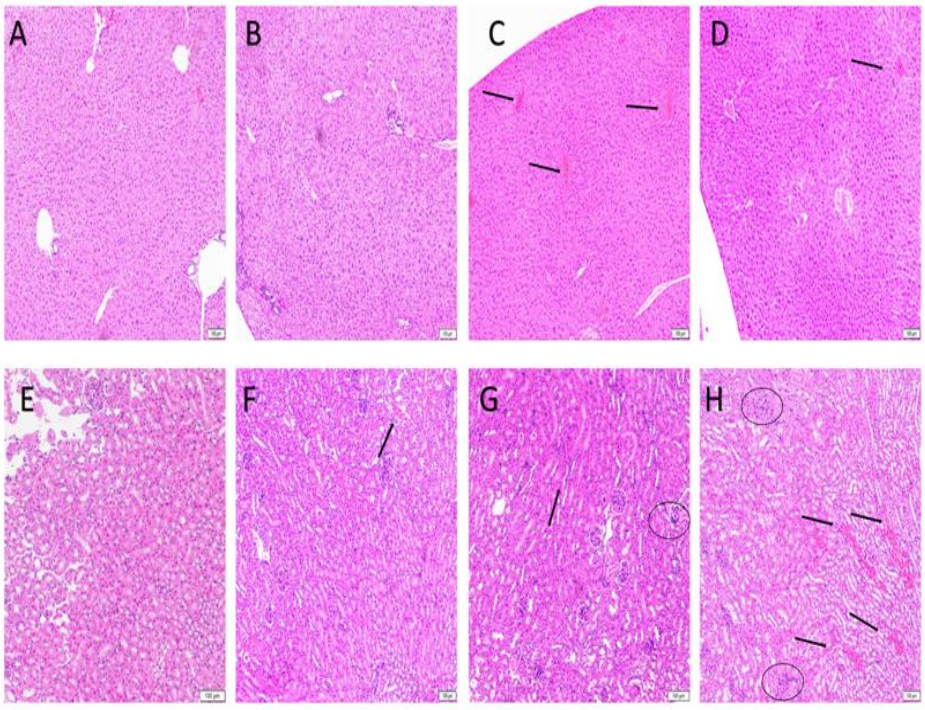

Figure 1: The histological effects of BPNLA treatment on liver and kidney tissues of mice. (A) The liver structure of the control group is normal, with no obvious lesions. (B) Interstitial tissue of the low-dose group is slightly enlarged, with mild bleeding; (C) Significant lesions occur in the middle-dose group, severe bleeding, and accompanied by inflammatory cell infiltration; (D) Interstitial tissue of the high-dose group is significantly expanded, with severe bleeding, hepatocytes appear vacuolated, and massive inflammatory cell infiltration. (E) Renal structure of the control group is normal, with no obvious inflammation; (F) Renal glomerulus of the low-dose group has mild bleeding, and interstitial tissue is slightly widened; (G) Renal interstitial tissue of the middle-dose group is significantly expanded, and inflammatory cell infiltration around glomerulus; (H) Severe

lesions occur in the high-dose group, extensive bleeding and massive inflammatory cell infiltration. The black arrows indicate hemorrhage, The circle indicates the infiltration of inflammatory cells. Scale bar=100 μm.

The impact of BPNLA on liver and kidney functions

Due to histological examination showing that BPNLA caused liver and kidney damage, we further detected liver and kidney function-related indicators. As shown in **Figure 2A-D**, compared with the control group, BPNLA increased the activity of ALT and AST in liver tissues and the levels of CRE and BUN in kidney tissues ($p < 0.05$), and the increase was gradually enhanced with the increase of BPNLA dose. The normality of blood lipids is also closely related to liver and kidney diseases. Therefore, we measured the levels of T-CHO, TG, TBA, and HDL-c in the blood. Compared to the control group, BPNLA treatment significantly increased the levels of T-CHO, TG, and TBA in serum, while HDL-c levels decreased (**Figure 2E-H**, $p < 0.05$). These results were consistent with our expectations, and combined with histopathological examination, indicated liver and kidney damage.

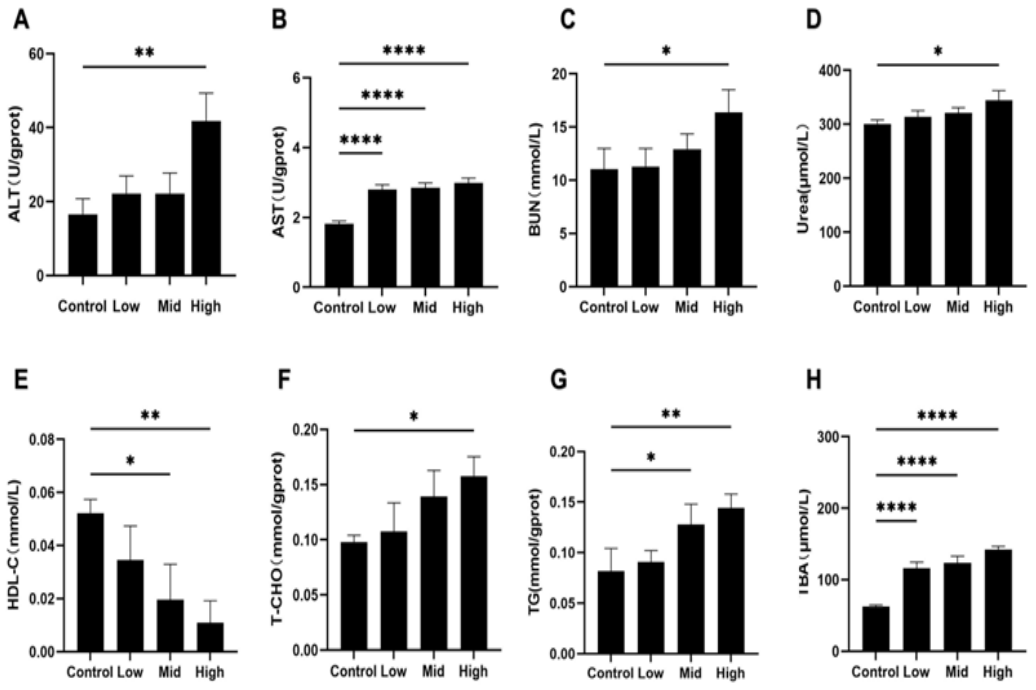

Figure 2: The effects of BPNLA exposure on biomarkers of liver and kidney function in mice. (A) Level of Alanine Aminotransferase (ALT); (B) Level of Aspartate Aminotransferase (AST); (C) Level of Blood Urea Nitrogen (BUN) ;(D) Level of creatinine (Urea); (E) Level of High-Density Lipoprotein Cholesterol (HDL-C) ;(F) Level of Total Cholesterol (T-CHO) ;(G) Level of Triglyceride (TG); (H) Level of Total Bile Acid (TBA). Control: saline; Low: 2.925 μg/kg/day; Mid: 146.25 μg/kg/day High: 7312.5 μg/kg/day. The data of this study are presented as mean ± SEM of three parallel measurements. Statistical significance (Control vs others: *, $P < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p<0.0001$).

The effect of BPNLA on oxidative stress parameters

Liver and kidney damage is often related to oxidative stress. To determine whether BPNLA causes oxidative damage, we examined the activities of SOD, GSH, and GPX as well as the content of MDA in liver and kidney tissue homogenates. The results showed that, compared to the control group, the activities of SOD, GSH, and GPX were significantly reduced after BPNLA treatment, while the content of MDA was significantly increased (**Figure 3**, $p < 0.05$).

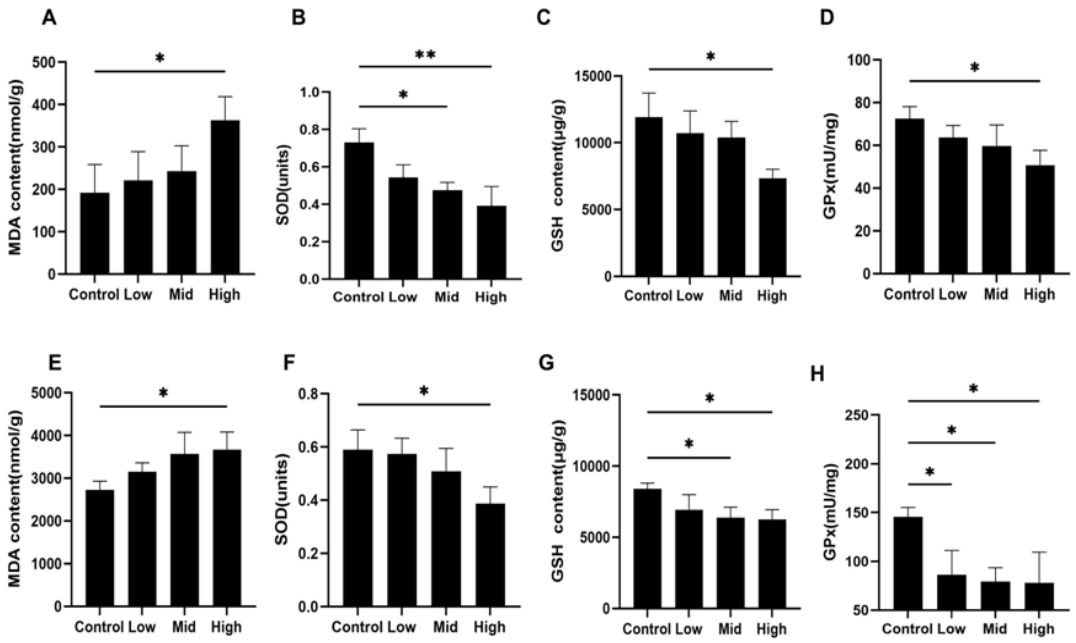

Figure 3: Effects of BPNLA exposure on oxidative stress markers in liver and kidney tissues of mice. (A-D) Changes in the contents of MDA, SOD, GSH, and GPX in liver tissues; (E-H) Changes in the contents of MDA, SOD, GSH, and GPX in kidney tissues. Control: saline; Low: 2.925 μg/kg/day; Mid: 146.25 μg/kg/day High: 7312.5 μg/kg/day. All values are expressed as the mean ± SEM of three independent experiments. Statistical significance (Control vs others: *, $P < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p<0.0001$).

BPNLA increased the expression of inflammatory markers in liver and kidney tissues

Since oxidative stress is often closely related to inflammation, we further detected the expression of inflammatory cytokines in the liver and kidney tissues of mice, the levels of inflammatory markers TNF-α and IL-1β were tested using ELISA kits. Consistent with the expected results, the obtained data showed that compared to the control group, BPNLA-treated mice induced inflammation by upregulating the levels of IL-1β and TNF-α in the liver and kidneys (**Figure 4A-D**, $p< 0.01$). However, there were differences between doses. Consistent with the ELISA results, gene expression experiments showed that compared to the control group, the mRNA levels of TNF-α and IL-1β in the liver and kidney tissues of the BPNLA group were significantly upregulated (**Figure 4E-H**, $p< 0.01$).

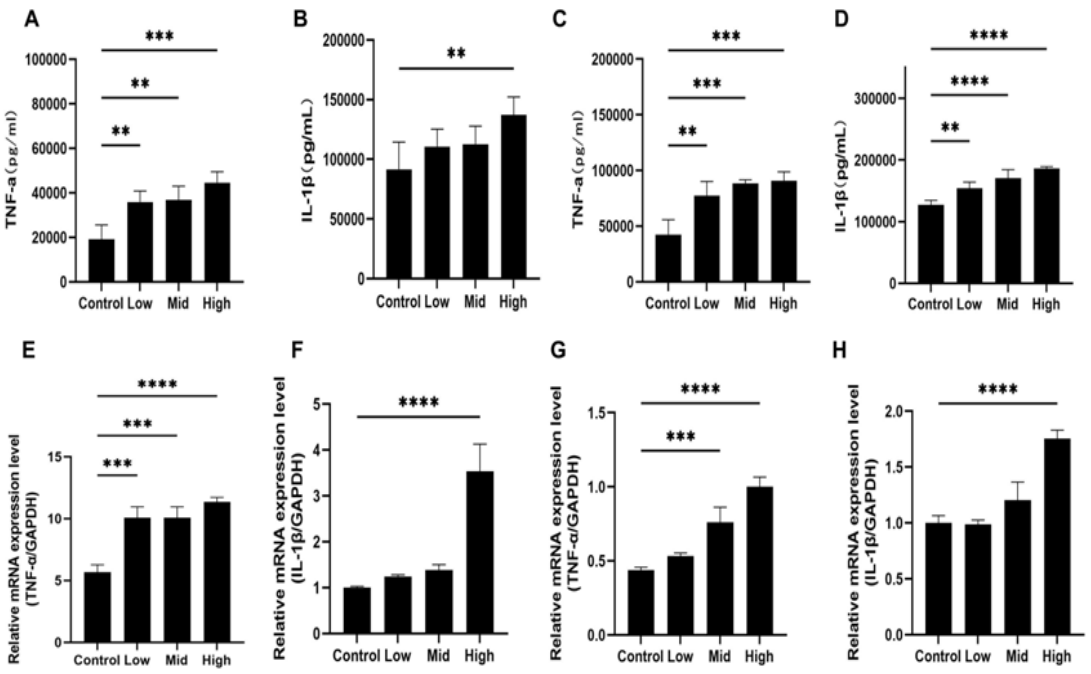

Figure 4: Effects of BPNLA exposure on inflammatory markers in liver and kidney tissues of mice. (A and B) Represent the relative mRNA expression levels of TNF-α and IL-1β in liver tissues. (C and D) Represent the relative mRNA expression levels of TNF-α and IL-1β in kidney tissues. (E and F) Represent the levels of TNF-α and IL-1β in the liver. (G and H) Represent the levels of TNF-α and IL-1β in the kidney. Control saline; Low: 2.925 μg/kg/day; Mid: 146.25 μg/kg/day; High: 7312.5 μg/kg/day. The data of this study are presented as mean ± SEM of three parallel measurements. Statistical significance (Control vs others: *, $P < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p<0.0001$).

Discussion

The liver and kidneys are the body's most important metabolic and excretory organs [15], they have numerous functions such as digestion, detoxification, and regulating the body's water balance. However, the liver and kidneys are the most common targets for the toxic effects of many foreign chemicals, including some pharmaceuticals [16]. The relationship between the liver and kidneys is often close, and changes in renal function can often be observed in patients with liver disease [17]. For example, cirrhosis often leads to acute kidney injury. When severe hepatitis occurs in the liver, it can also induce abnormalities in the kidneys, manifested as hepatorenal syndrome [18]. Previous studies have reported that BPNLA has toxic effects on the lungs and nervous tissues [10], but its toxicity on the liver and kidney tissues is unclear. We established an *in vivo* toxicity model by gavage of mice for 35 days, aiming to investigate the liver and kidney injury effects of BPNLA in mice. Transaminases, including AST and ALT, catalyze the transfer of amino groups between amino acids and ketones [19]. Under the influence of pathogenic factors, degeneration and necrosis of liver cells occurs, leading to increased permeability of the cell membrane and the release of ALT and AST into the bloodstream, which in turn results in increased transaminase activity [20]. As ALT is mainly found in the liver, it is a specific marker for liver damage, whereas AST can also be detected in the kidneys, heart, and skeletal muscle, making it

less specific [21]. Our results showed that in a mouse model with BPNLA-induced liver and kidney injury, the activity of ALT and AST increases, indicating the occurrence of liver injury. The morphological damage to the liver tissue further emphasized this. The levels of urea and creatinine are commonly used to determine kidney damage [22]. Urea is the end product of protein metabolism and is mainly excreted by the kidneys. It is filtered by the glomeruli, partially reabsorbed by the renal tubules, and excreted in small amounts [23]. Creatinine is a metabolic product of creatine phosphate in muscle, and it is almost completely filtered by the glomerulus, making it a more representative marker of kidney damage [24]. An increase in both values indicates a decrease in the glomerular filtration rate [25]. In the experimental group, the creatinine and urea levels of the mice increased significantly, indicating that BPNLA induces glomerular damage. In addition, the tissue pathology of BPNLA-induced renal injury showed severe deterioration of renal structure. At the same time, compared with the control group, BPNLA increased the levels of T-CHO, TBA, and TG, in the serum of mice, while reducing HDL-c. Studies have shown that low HDL-c is an important risk factor for the occurrence of renal dysfunction, and chronic kidney disease is often accompanied by hypertriglyceridemia. As the main metabolic site of lipoproteins, the liver plays an important role in regulating cholesterol. In addition, TBA can also be used to evaluate liver injury [26-29]. This indicated that the toxic effects of BPNLA may have interfered with hepatorenal metabolism, and these changes in parameters may originate from free radical effects. Because the increase in serum cholesterol often accelerates the production of free radicals, which leads to oxidative stress [30]. Oxidative stress refers to a state of imbalance between oxidation and antioxidant effects in the body, which is more prone to oxidation [31]. Reactive Oxygen Species (ROS) are the main cause of oxidative stress. Under physiological conditions, ROS, as natural by-products of oxygen metabolism, are at a low level in the body and can promote immunity, repair, survival, growth, etc. However, when some exogenous substances stimulate the production of excessive ROS, they can cause oxidative damage to cellular macromolecules such as DNA, proteins, and lipids, leading to cell necrosis [32,33]. In this study, the production of ROS was evaluated by measuring the antioxidant status of cells and the level of MDA, a biomarker for oxidative stress. The results showed that BPNLA caused significant oxidative stress in liver and kidney tissues, manifested as increased MDA levels, decreased antioxidant enzyme activity (SOD, GPX), and GSH levels. SOD and GPX are two essential antioxidants that can destroy toxic peroxides and protect cells [34]. Therefore, the activity of SOD and GPX indirectly reflects the ability to eliminate free radicals. GSH is a specific substance for detoxification and is the main intracellular antioxidant and free radical scavenger. It is also the substrate of the antioxidant enzyme GPX [35]. MDA is one of the most important products of membrane lipid peroxidation, the significant increase in MDA concentration reflects the rate and intensity of lipid peroxidation in the body [36]. Oxidative stress can also activate inflammatory responses, causing infiltration of inflammatory cells and the release of inflammatory mediators [37]. Among the types of inflammatory injury affecting internal organs, IL-1β is one of the earliest pro-inflammatory cytokines [38]. TNF-α is a pro-inflammatory cytokine that has pleiotropic effects on various cell types and mainly mediates the acute phase response [39]. The synergistic action of IL-1β and TNF-α activates NF-κB in cells, induces inflammatory responses, promotes granulocyte aggregation, and leads to tissue injury. In the current study, compared with the control group, penicillamine significantly increased the levels of TNF-α and IL-1β.

However, this study has some limitations. First, we established three doses of administration in this study. The low dose was calculated based on the acceptable daily dose of BPNLA for humans in combination with the

conversion factor between humans and mice [40]. Because BPNLA was administered orally for only 35 days in this study, it was impossible to simulate the cumulative toxic effects of long-term low-dose exposure to BPNLA in mice. However, humans often take antibiotics for long periods, which can cause high doses of BPNLA to accumulate in the body. Therefore, we increased the low dose by 50- and 2500-fold to serve as the medium and high dose groups, respectively, to simulate the toxic effects of long-term low-dose exposure. Our results showed that all three dose groups exhibited different degrees of liver toxicity, with the high-dose group showing higher toxicity and significant changes in various indicators. This suggested that years of accumulation of antibiotics may not only lead to drug resistance but also pose potential risks to the liver and kidneys, which is a warning for our health. Secondly, this study only constructed an *in vivo* model to evaluate toxic effects. Future studies should continue to investigate *in vitro* cell lines and explore the mechanisms of action in more detail. Third, this study only measured toxicity in specific tissues of mice (liver, kidney, and serum), which may limit the comprehensive evaluation of BPNLA toxicity. Therefore, future studies should include a broader range of tissues for a more comprehensive assessment of BPNLA toxicity.

Conclusion

In conclusion, our research showed that long-term exposure to low doses of BPNLA may accumulate in liver and kidney tissue, induce oxidative stress, and cause liver and kidney toxicity, this suggested that penicillin residues and heat treatment in meat products are a risk factor for human health. Given the critical role of oxidative stress in liver disease, the application of antioxidants to treat BPNLA-induced liver and kidney disease could be a promising treatment strategy in the future.

Author Contributions

Ruixue Hu drafted the initial writing and data curation, Jian Guo reviewed and edited the final manuscript, Shiying Lu and Yansong Li performed formal analysis, Zengshan Liu secured funding, Ke Zhao conducted the investigation, Pan Hu established the methodology, Yang Wang managed the project, Honglin Ren supervised the process.

Institutional Review Board Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Acknowledgments

We thank the staff of the Natural Science Foundation of Jilin Province (No. 20210101012JC) for their support.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have potentially influenced the work reported in this paper.

References

1. Chen J, GG Ying, WJ Deng. Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns. J Agric Food Chem. 2019;67(27):7569-86.
2. Chen YL, et al. High sensitive detection of penicillin G residues in milk by surface enhanced Raman scattering. Talanta. 2017:167:236-41.
3. van den Bogaard AE, EE Stobberingh. Antibiotic usage in animals - Impact on bacterial resistance and public health. Drugs. 1999;58(4):589-607.
4. Wilkinson J, A Zainal, SY Naqvi. Penicillin-induced liver injury during treatment for ocular neurosyphilis. BMJ Case Rep. 2016:2016:bcr2016215821.
5. Wang HX, et al. Antibiotic Body Burden of Chinese School Children: A Multisite Biomonitoring-based Study. Environ Sci Technol. 2015;49(8):5070-9.
6. Padari H, et al. Pharmacokinetics of Penicillin G in Preterm and Term Neonates. Antimicrob Agents Chemother. 2018;62(5):e02238-17.
7. Musser JMB, KL Anderson, JO Boison. Tissue disposition and depletion of penicillin G after oral administration with milk in unweaned dairy calves. J Am Vet Med Assoc. 2001;219(3):346-50.
8. Cui C, et al. Acute and chronic toxicity assessment of benzylpenicillin G residue in heat-treated animal food products. Chemosphere. 2018 ul:202:757-67.
9. Birner J. Determination of phenoxymethyl penicilloic acid and phenoxyethyl penicilloic acid in urine in the presence of the parent penicillins. J Pharm Sci. 1970;59(6):757-60.
10. Cui C, et al. A preliminary investigation of the toxic effects of Benzylpenicilloic acid. Food Chem Toxicol. 2018:111:567-77.
11. Ahmed ZSO, et al. Protective effect of starch-stabilized selenium nanoparticles against melamine-induced hepato-renal toxicity in male albino rats. Int J Biol Macromol. 2021:191:792-802.
12. Maalej A, et al. Olive phenolic compounds attenuate deltamethrin-induced liver and kidney toxicity through regulating oxidative stress, inflammation and apoptosis. Food Chem Toxicol. 2017;106(A):455-65.
13. Ge JL, et al. Secoisolariciresinol diglucoside mitigates benzo [a] pyrene-induced liver and kidney toxicity in mice via miR-101a/MKP-1-mediated p38 and ERK pathway. Food Chem Toxicol. 2022:159:112733.
14. El-Moselhy MA, AAK El-Sheikh. Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed Pharmacother. 2014;68(1):101-10.
15. Sangodele JO, et al. The para isomer of dinitrobenzene disrupts redox homeostasis in liver and kidney of male wistar rats. Biochem Biophys Rep. 2017:10:297-302.
16. Guo H, et al. Alleviation of doxorubicin-induced hepatorenal toxicities with sesamin via the suppression of oxidative stress. Hum Exp Toxicol. 2016;35(11):1183-93.
17. Roedl K, JD, Fuhrmann V. [Organinteraction between liver and kidney]. Dtsch Med Wochenschr. 2017;142(18):1365-72.
18. Gupta K, et al. Acute kidney injury and hepatorenal syndrome in cirrhosis. World J Gastroenterol. 2021;27(26):3984-4003.
19. Sookoian S, CJ Pirola. Liver enzymes, metabolomics and genome-wide association studies: From systems biology to the personalized medicine. World J Gastroenterol. 2015;21(3):711-25.

20. Xu L, et al. Protective Effects of Taraxasterol against Ethanol-Induced Liver Injury by Regulating CYP2E1/Nrf2/HO-1 and NF-κB Signaling Pathways in Mice. Oxid Med Cell Longev. 2018:2018:8284107.
21. Abou-Zeid SM, EA Tahoun, HO AbuBakr. Ameliorative effects of jojoba oil on fipronil-induced hepatorenal- and neuro-toxicity: the antioxidant status and apoptotic markers expression in rats. Environ Sci Pollut Res Int. 2021;28(20):25959-71.
22. Peng X, et al. A Machine Learning-Based Prediction Model for Acute Kidney Injury in Patients With Congestive Heart Failure. Front Cardiovasc Med. 2022:9:842873.
23. Chen XY, et al. Protective effect of berberine on doxorubicin-induced acute hepatorenal toxicity in rats. Mol Med Rep. 2016;13(5):3953-60.
24. Borges LP, et al. Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. Toxicology. 2005;210(1):1-8.
25. Gur C, et al. Chemopreventive effects of hesperidin against paclitaxel-induced hepatotoxicity and nephrotoxicity via amendment of Nrf2/HO-1 and caspase-3/Bax/Bcl-2 signaling pathways. Chem Biol Interact. 2022:365:110073.
26. Brock WJ, et al. Bile Acids as Potential Biomarkers to Assess Liver Impairment in Polycystic Kidney Disease. Int J Toxicol. 2018;37(2):144-54.
27. Huang JK, HC Lee. Emerging Evidence of Pathological Roles of Very-Low-Density Lipoprotein (VLDL). Int J Mol Sci. 2022;23(8):4300.
28. Elsayed A, et al. Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats. Sci Rep. 2021;11(1):13979.
29. Zhong JY, HC Yang, V Kon. Kidney as modulator and target of "good/bad" HDL. Pediatr Nephrol. 2019;34(10):1683-95.
30. Huang S, et al. Hypolipidemic and Antioxidant Effects of Malus toringoides (Rehd.) Hughes Leaves in High-Fat-Diet-Induced Hyperlipidemic Rats. J Med Food. 2017;20(3):258-64.
31. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015:4:180-3.
32. Yang SS, GJ Lian. ROS and diseases: role in metabolism and energy supply. Mol Cell Biochem. 2020;467(1-2):1-12.
33. Moloney JN, TG Cotter. ROS signalling in the biology of cancer. Semin Cell Dev Biol. 2018:80:50-64.
34. Su LJ, et al. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxid Med Cell Longev. 2019:2019:5080843.
35. Eldaim MAA, et al. Ginseng attenuates fipronil-induced hepatorenal toxicity via its antioxidant, anti-apoptotic,and anti-inflammatory activities in rats. Environ Sci Pollut Res Int. 2020;27(36):45008-17.
36. Gao HT, et al. Dietary restriction reduces blood lipids and ameliorates liver function of mice with hyperlipidemia. J Huazhong Univ Sci Technolog Med Sci. 2017;37(1):79-86.
37. Hussain T, et al. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? Oxid Med Cell Longev. 2016:2016:7432797.
38. Yazdi AS, K Ghoreschi. The Interleukin-1 Family. Adv Exp Med Biol. 2016:941:21-29.
39. van Loo G, MJM Bertrand. Death by TNF: a road to inflammation. Nat Rev Immunol. 2023;23(5):289-303.

40. Nair A, S Jacob. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31.

Citation of this Article

Hu R, Guo J, Zhao K, Ren H, Liu Z, Lu S, Li Y, Wang Y and Hu P. Chronic Exposure to Subtherapeutic Levels of Benzylpenicilloic Acid Mediates Hepatorenal Dysfunction in Murine Models. Mega J Case Rep. 2024;7(2):2001-2013.

Copyright