



Effects of Cadmium and/or Lead on Autophagy and Liver Injury in Rats

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Abstract

Exposure to cadmium (Cd) and lead (Pb) can induce liver damage. However, the effects of the combined exposure to Cd and Pb on liver function have not been fully clarified. In the present study, we investigated the liver function in rats co-exposed to Cd and Pb. A total of 24 female SD rats were divided into 4 groups as follows: control group (DDW), Cd group (50 mg/l Cd), Pb group (300 mg/l Pb), Pb + Cd group (300 mg/l + 50 mg/l Cd). Following 12 weeks of continuous exposure, the results showed a large accumulation of Cd and Pb in the liver. The Liver weight and Liver coefficient were decreased, as well as liver structure and function was destroyed. In addition, Pb + Cd group exhibited additional pathological alterations. Moreover, the indices of oxidative stress and related trace elements were detected following treatment. The results showed that the single treatment of Pb or Cd and the combined Cd and Pb treatment could upregulate the contents of antioxidant enzymes and related trace elements. We further examined the expression levels of autophagy-related proteins and mRNAs, and we found that the single treatment of Pb or Cd and the combined Cd and Pb treatment could upregulate the expression of levels of autophagy-related proteins and mRNAs (*Atg5*, *Atg7*, *Beclin-1*, *p62*, and *LC3*). Transmission electron microscopy revealed the presence of autophagosomes in the exposed groups. All the results indicated that Cd and Pb may affect the level of oxidative stress and autophagy in hepatocytes, whereas the combination of Cd and Pb showed a tendency of escalation compared with the single treatment group.

Keywords Cadmium and lead · Oxidative stress · Liver · Autophagy

Introduction

Previous studies have shown that cadmium (Cd) and lead (Pb) exposure can cause damage to various organs. Liver and kidney are the main target organs [1]. The biological half-life of Cd in the human and animal organisms is estimated from 10 to 30 years. Once Cd enters the body, its excretion is considerably difficult [2]. With the recent research, it is shown that the workers in pigment and batteries production, galvanization and recycle ng of electric tools, and related electronics industries may consume too much lead and/or cadmium [3–5]. Both Cd and Pb can cause liver damage in a variety of ways.

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Previous studies have shown that both Cd and Pb exposure can lead to hepatocyte enlargement, inflammatory cell infiltration [6], and hepatocyte necrosis, whereas it can also cause elevation in the plasma activity levels of liver enzymes, such as aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) [7]. Furthermore, Pb can also affect the production of heme, destroy the structure of the cell membrane, cause lipid metabolic dysfunction, and reduce liver detoxification function [8].

The mechanism of Cd or Pb-mediated toxicity has been widely studied, among which oxidative stress is considered as an important molecular attribute to their cytotoxicity. Under normal conditions, the redox system of the body is in a state of dynamic equilibrium. The antioxidant enzymes play an important role in maintaining the body's redox system. The entrance of Cd or Pb into the body results in a change of the concentration of a variety of trace elements [9], and it can also combine with the SH groups affecting the activity of a variety of proteins and enzymes [10], such as antioxidant enzymes.

Autophagy is a biological process in which eukaryotic cells use lysosomes to degrade organelles and proteins [11]. By

transporting damaged organelles and misfolded proteins to lysosomes for degradation, the cells can adapt to their nutritional requirements. Autophagy plays an important role in maintaining the number and function of organelles and stabilizing the intracellular environment [12]. The mechanism of autophagy is relatively complex and is regulated by various genes. These autophagy genes play an important role in the process, which is divided into four stages as follows: autophagy initiation, autophagosome extension, autophagosome maturation, and autophagosome degradation [13, 14]. In the physiological state, autophagy is inhibited and maintained at a low level. When the cells are stimulated by external stimuli, such as heavy metals, radiation and harmful compounds, the autophagy levels are significantly upregulated. Previous studies have shown that both Pb and Cd can induce autophagy in cells [15, 16]. During the initiation of autophagy, in addition to the mTOR and ULK complexes, Beclin1 can combine with PI3K, PK3C3, and with other proteins in order to promote the initiation of autophagosome formation. Moreover, the PI3K complex is further associated with the maturation of autophagosomes [17]. The apoptosis-stimulating protein 2 (ASPP2) negatively regulates autophagosome maturation by interfering with the formation of the PI3K complex and by regulating Beclin1 transcription [18]. The extension of autophagosomes is involved in various autophagy-associated proteins. Atg7 activates Atg12 and forms complexes with Atg5 and Atg16L, which recruit microtubule-associated protein light chain 3 (LC3) and promote the extension of autophagosomes [19].

Our previous studies have shown that Pb and Cd can promote the generation of ROS and induce autophagy. However, the effects of the combined exposure to these chemicals on liver oxidative stress of rats with regard to the induction of autophagy are unclear.

Materials and Methods

Chemicals and Antibodies

Lead acetate (PbAc₂), cadmium acetate (CdAc₂), and anti-LC3B were obtained from Sigma-Aldrich (St. Louis, MO, USA), whereas anti-Beclin 1, Atg5, Atg7, P62, and β -actin antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The enhanced chemiluminescence (ECL) detection kit was purchased from Millipore (Burlington, MA, USA). Oligonucleotide primers were synthesized by Invitrogen (Shanghai, China). PrimeScriptTM RT Reagent kit and SYBR[®] Premix Ex Taq were obtained from TaKaRa (Dalian, China). All the transferase detection kits were purchased from Nanjing Jiancheng Bioengineering Institute

(Nanjing, China). The rest of the reagents were of analytical grade or the highest quality available.

Animals and Treatment

A total of 24 female Sprague-Dawley rats weighing 80–100 g were obtained from the Laboratory Animal Center of the Jiangsu University (Zhenjiang, China). The animals were housed individually on a 12 h light/dark cycle with unlimited standard rat food and double-distilled water (DDW). All experimental procedures were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council and were approved by the Animal Care and Use Committee of the Yangzhou University (Approval ID: SYXK (Su) 2017–0044). All surgeries and operations were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize any suffering experienced by the animals used in this study. The animals were divided randomly into four groups: A total of 6 rats in each group were fed in separate cages as follows: control group (DDW), cadmium group (50 mg/l Cd), lead group (300 mg/l Pb), and cadmium + lead group (300 mg/l Pb + 50 mg/l Cd). According to the grouping used, the rats consumed DDW solution of 300 mg/l Pb and 50 mg/l Cd in their drinking water. All rats were sacrificed by cervical dislocation 12 weeks following initial treatment.

Hepatotoxicity Analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the serum were measured by specific commercial kits (Catalog No. C009–1 for ALT and C010–1 for AST) used from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The assays were conducted according to the manufacturer's instructions.

Detection of Element Contents in Liver Tissues

The liver tissue samples were dried at 80 °C for 12 h. A total of 200 mg dried samples were dissolved in 6 ml concentrated nitric acid, and the samples were digested in a microwave digestion instrument (Multiwave GO, Anton Paar, Austria). The digested samples were diluted in double-distilled water, and the Cd and Pb levels were detected in the liver tissues by plasma optical emission spectrophotometry (ICP-OES) (Optima 7300 DV, PerkinElmer, USA).

Histopathology

Tissue specimens of the liver were fixed in 10% buffered formalin at 4 °C for 24 h, dehydrated in a series of ethanol solutions, and subsequently immersed in xylene and embedded in paraffin. The tissues that were approximately 4 μ m in

thickness were sectioned from paraffin blocks and stained with hematoxylin and eosin (H&E). Histological sections were examined, and the photos were obtained under a fluorescence microscope (Leica 2500, Leica Corporation, Germany).

Detection of Oxidative Stress-Related Indices

Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities were measured in the liver tissues by specific commercial kits (Catalog No. A001–3, A005, A007–1-1, respectively) from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instruction. The levels of glutathione (GSH) and malondialdehyde (MDA) in the liver tissues were measured with commercial kits from the same company (Catalog No. A006–2 for GSH and A003–1 for MDA).

Western Blot Analysis

Liver proteins were extracted using the radio-immunoprecipitation assay lysis buffer and a protease inhibitor cocktail. The extraction was performed on ice for 30 min and was completed by ultrasonication (60 Hz for 5 s, 4 times). The homogenates were centrifuged for 10 min (1500 g, 4 °C). Following centrifugation, the protein concentration was assayed using a bicinchoninic acid protein assay kit (Beyotime, Jiang Su, China). Western blotting was performed as previously described [20]. Densitometric analysis was performed by the Image Lab software (Bio-Rad, Hercules, CA, USA). The protein levels were determined by standard scanning densitometry and subsequently normalized to the corresponding density of β -actin.

Transmission Electron Microscopy

The cell ultrastructure and percentage of cell autophagy of the rat hepatocytes were observed using transmission electronic microscopy (Philips CM-100, Holland). The harvested liver was fixed in 2.5% glutaraldehyde at 4 °C for 12 h. Subsequently, the samples were treated with 1% osmium tetroxide for 2 h at room temperature. Following dehydration with a graded ethanol series, the samples were placed in pure acetone and Epon 812 epoxy solution for 30 min and subsequently embedded in Epon 812. The ultrathin sections were cut with a diamond knife, stained with aqueous uranyl acetate and lead citrate, and observed in a transmission electron microscope.

RNA Extraction, Reverse Transcription, and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from liver tissues using the Trizol reagent, and subsequently, approximately 900 ng RNA was

reverse transcribed according to the manufacturer's instructions (Takara, Japan). The primers for *Atg5*, *Atg7*, *Beclin-1*, *p62*, *LC3*, and β -actin are listed in Table 1. Gene expression levels were measured using a real-time PCR system (Applied Biosystems 7500, USA). The reaction was performed with a SYBR® Premix Taq™II kit (Takara, Japan). The analysis of the relative mRNA levels was performed as described in a previous study [21].

Statistical Analysis

The data in the present study are presented as the mean \pm standard deviation (SD). Statistical data comparisons between groups were evaluated by one-way ANOVA using the SPSS 22.0 statistical software (SPSS, Chicago, USA). A $p < 0.05$ was considered for significant difference, and a $p < 0.01$ was considered for highly significant differences.

Results

Effects of cd and Pb on Liver Weight and on the Liver Coefficients

Following treatment, the liver weight and liver coefficient were measured. As shown in Fig. 1a, the liver weight was not significantly different between the control and the Cd groups, while the liver weight in the Pb and Pb + Cd groups was significantly decreased compared with that of the control group. Moreover, no significant difference was noted in the liver weight between the Pb + Cd and the Cd or Pb groups. As also shown in Fig. 1b, the liver coefficient was not significantly different between the control and the Cd groups, while the liver coefficient in the Pb and Pb + Cd groups was significantly decreased compared with that of the control group and compared with the Cd group, the Pb+Cd groups was significantly decreased.

Assessment of Liver Function and cd and Pb Contents in the Liver Tissue

Liver function was evaluated, and Pb accumulation and Cd accumulation were detected in the liver. The results are shown in Fig. 1. The activities of the ALT and AST enzymes in the Cd- and Pb-treated groups alone or in combination were significantly higher than those noted in the control group. Moreover, the activities of ALT and AST of the combined treatment group were significantly higher than those of the single treatment Pb and Cd groups. In addition, AST indicated higher levels in the serum compared with those of ALT (Fig. 1c, d). The results of the Cd and Pb contents are shown in Fig. 1e and f. Cd accumulation in the Cd group was significantly higher than that noted in the control group, whereas Cd accumulation in the Pb + Cd group demonstrated no

Table 1 Primer sequences of target genes with their corresponding PCR product size

Gene	Upstream (5'-3')	Downstream (5'-3')	Length(bp)
<i>β-actin</i>	CGTTGACATCCGTAAAGACCTC	TAGGAGCCAGGGCAGTAATCT	110
<i>Atg7</i>	GCTGGTCTCCTTGCTCAAC	CAGGGTGCTGGGTTAGGTTA	101
<i>Atg5</i>	AGGCTCAGTGGAGGCAACAG	CCCTATCTCCCATGGAATCTTCT	72
<i>Beclin-1</i>	TTCAAGATCCTGGACCGAGTGAC	AGACACCATCCTGGCGAGTTTC	142
<i>P62</i>	TATTACAGCCAGAGTCAAGG	CTACATACAGAAGCCAGAATG	358
<i>LC3</i>	ACGGCTTCTGTACATGGTC	GTGGGTGCCTACGTTCTGAT	171

significant difference compared with that of the Cd group. Pb treatment indicated similar results to those of the Cd treatment.

Cd and Pb Induced Histopathological and Ultrastructural Damage in the Liver Tissue

The alterations in the histopathological structures of the liver were observed following treatment. As shown in Fig. 2a, the hepatic cells in the Cd group were arranged in a disordered way. The cells exhibited degenerative and necrotic features that were indicative of microvesicular steatosis. In the Pb group, the hepatic cells exhibited similar pathological changes. In addition, hyperemia in the hepatic sinusoid was noted. The hepatic cells in the Pb + Cd group exhibited serious pathological alterations, including vacuolization, reduced cytoplasmic volume, and nuclear rupture and disappearance.

The morphological characteristics of the hepatic cells were assessed using electron microscopy and were found regular

with nearly circular nuclei, well-distributed cytoplasm, clear presence of nucleoli, and abundant normal size mitochondria. Nuclear deformation was evident with nuclear chromatin in the outer nuclear layer, and an uneven distribution of these structures was noted. Marked nuclear condensation was present which was accompanied by a decrease in the number of mitochondria and mitochondrial swelling. These changes were observed in hepatic cells following Cd or Pb exposure. Combined treatment with Cd and Pb increased the ultrastructural damage noted in the hepatic cells (Fig. 2b).

Effects of cd and Pb on Oxidative Stress Biomarkers and on the Antioxidant-Related Trace Elements in the Liver Tissues

We detected the oxidative stress levels following treatment of the animals with Cd and Pb, as shown in Fig. 3a-e. Exposure of the animals to these chemicals caused no effect on SOD activity compared with the corresponding levels noted in the

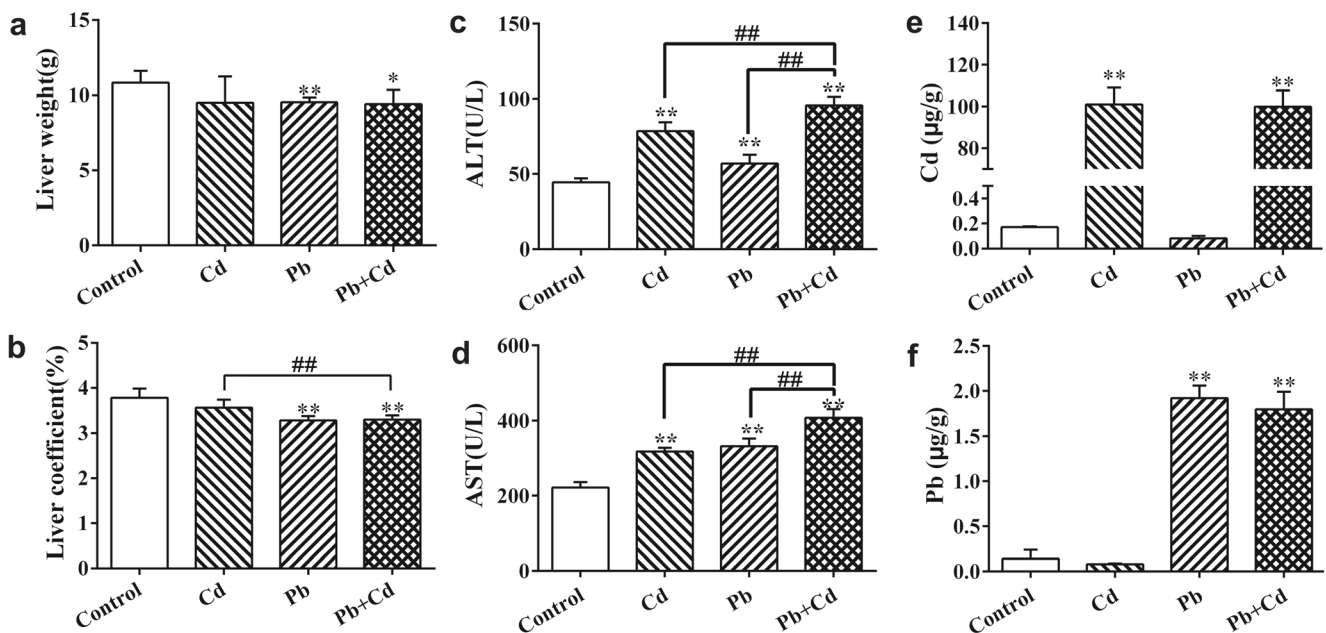


Fig. 1 Effects of Cd and/or Pb injury on liver tissues and determination of Cd and Pb contents in these tissues. To detect liver injury, we measured the parameters liver weight (a), liver coefficient (b) and liver function

(ALT (c), AST (d)). We further tested the levels of Pb (f) and Cd (e) in the liver tissue. * $P < 0.05$, ** $P < 0.01$; # $P < 0.05$, ## $P < 0.01$

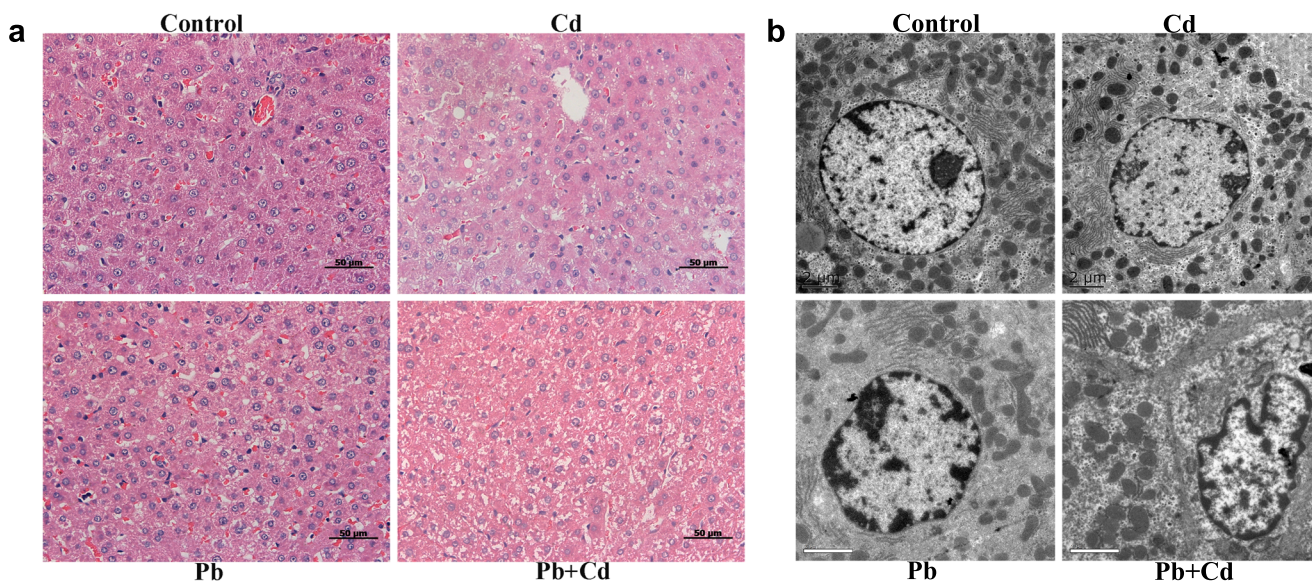


Fig. 2 Effects of Cd and/or Pb on histopathology and ultrastructure of the liver. The effects of Cd and/or Pb on liver histopathology and ultrastructure were observed by H&E staining (a) and by TEM (b), respectively

control group, while the combined treatment of Cd with Pb decreased SOD activity significantly compared with that of the Pb group (Fig. 3a). The content of MDA was significantly increased in the Cd group and significantly decreased in the Pb and Pb + Cd groups, respectively, compared with those of

the control group. In addition, the combined treatment of Cd with Pb further reduced MDA content compared with that of the Pb treatment (Fig. 3b). Cd and/or Pb exposure significantly increased CAT activity compared with that of the control group, whereas the content of CAT in the Pb + Cd group was

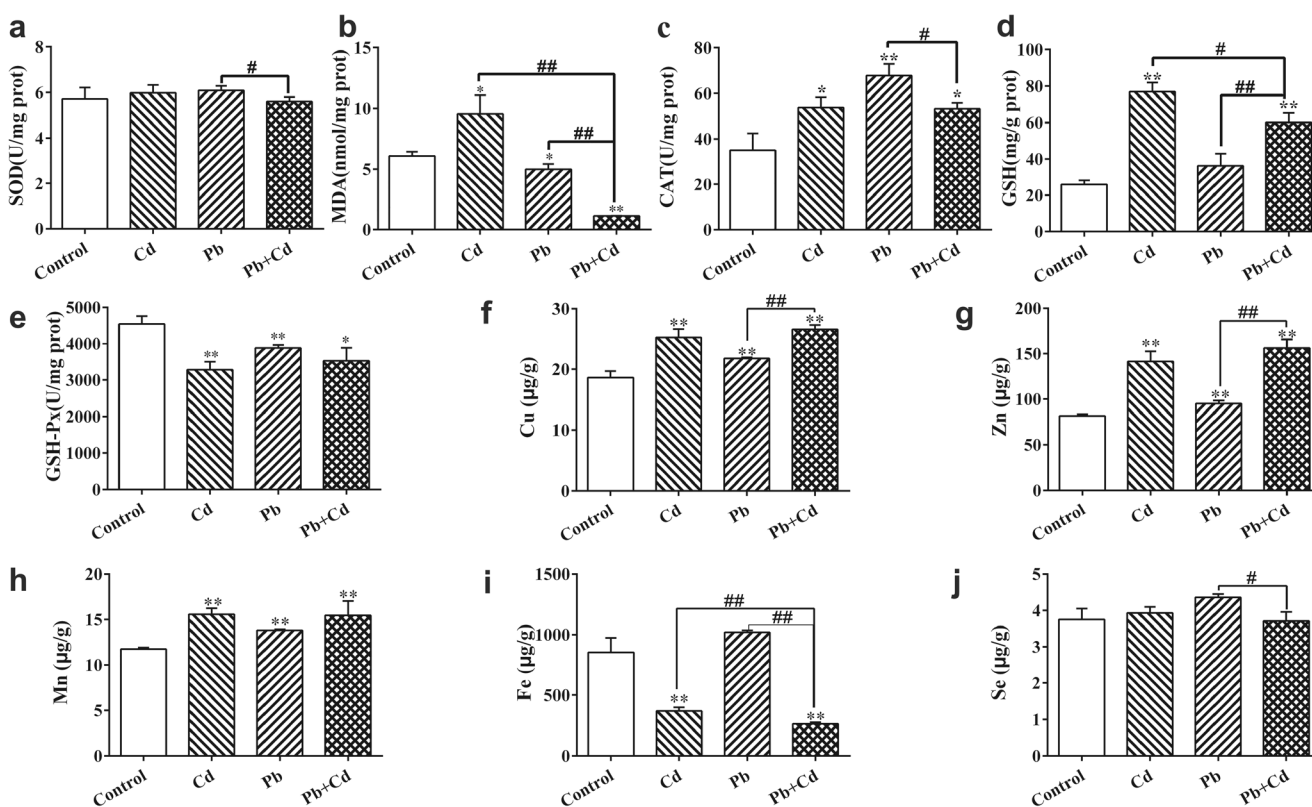


Fig. 3 Effects of Cd and/or Pb on oxidative stress biomarkers and antioxidant-related trace elements in liver. The content of MDA (b), SOD (a), CAT (c), GSH (d), and GSH-Px (e) was detected. Detection

of trace elements related to antioxidant enzymes such as Cu (f), Zn (g), Mn (h), Fe (i), and Se (j). * $P < 0.05$, ** $P < 0.01$; # $P < 0.05$, ## $P < 0.01$

significantly lower than that of the Pb group (Fig. 3c). The content of GSH in the Cd group was significantly higher than that of the control group, while no significant difference was noted between the Pb and the control groups. The combined treatment of Cd with Pb alleviated the increase in the GSH content caused by cadmium treatment alone (Fig. 3d). We further detected GSH-Px activity following treatment, and it appeared to exert the opposite effect compared with that on the GSH-Px activity and GSH content in the liver (Fig. 3e).

The concentrations of Cu, Zn, and Mn in the experimental groups were significantly higher than those in the control group, while the concentration levels of Cu and Zn in the Pb + Cd group were significantly higher than those in the Pb group (Fig. 3f-h). The Fe levels were significantly decreased following Cd exposure and significantly increased following Pb exposure compared with those of the control group. In addition, a highly significant difference was noted between the combination treatment group and the single Cd or Pb treatment group (Fig. 3i). The content of Se in the Pb group was significantly higher than that of the Pb + Cd group, whereas no significant difference was present between the Pb and control groups (Fig. 3j).

Effects of cd and Pb on the Levels of Autophagy in the Liver

We evaluated the levels of autophagy following treatment, as shown in Fig. 4 and observed the increase in the expression levels of Beclin1, Atg7, p62, Atg5, and LC3II in the experimental groups compared with those of the control group. In addition, the expression levels of all these autophagy-related proteins in the Pb + Cd group were significantly higher than those noted in the single treatment of Cd or Pb. In addition, we detected the expression levels of autophagy-related genes, as shown in Fig. 5a-e. The levels of the autophagy-related mRNAs in the experimental groups were significantly higher than those in the control group. The only difference noted was that the levels of these autophagy-related mRNAs in the Pb + Cd group were not higher than those of the Cd or Pb single treatment groups (Fig. 5e). These levels were significantly lower than those noted in the Pb single treatment group. Finally, we observed the levels of autophagy by transmission electron microscopy. Higher percentage of autophagy was noted in the treatment groups compared with that noted in the control group (Fig. 5f).

Discussion

Various harmful substances and microorganisms, such as heavy metals, viruses, and bacteria, cause their side effects in the liver, by stimulating cell damage and causing cell necrosis, apoptosis, inflammation, and autophagy. This

eventually leads to liver diseases. Cd and Pb can be accumulated in human organism by various ways. A large number of compelling evidence demonstrates that Cd and Pb are closely associated with several types of liver diseases [22]. In addition, Cd and Pb exposure can lead to tumor development [23].

Accumulating incidence suggests that the increase in the organ weight may be caused by pathological changes, such as cell swelling, edema, congestion, hemorrhage, and hypertrophy, whereas the decrease in the organ weight may be caused by pathological changes, such as dehydration, atrophy, necrosis, and decay. Long-term exposure to poisons can lead to loss of appetite or digestive dysfunction, resulting in slow weight gain or weight loss, as well as organ weight loss [24]. Yuan et al. found that the low-dose combination of Cd and Pb exposure exhibited no significant effects on the examined organ coefficients (including liver, brain, and lung) compared with those of the control group [25]. An increase was also noted in the levels of ALT and AST in the serum, which are indicators of liver dysfunction [26]. Previous studies demonstrated that the activities of ALT and AST were decreased following exposure to Cd and Pb mixtures [27]. In the present study, the changes in the liver tissue weight were monitored, by calculating the relative liver weights as liver coefficient based on the ratio of liver weight to body weight [28]. This was used as the serum biochemical index following treatment of rats with Pb and Cd. Pb treatment decreased the liver coefficient, whereas treatment with both Cd and Pb increased serum ALT and AST levels. In addition, the combined treatment of Cd with Pb could further increase serum ALT and AST levels. The concentration of Pb in the drinking water of the treatment group was 6 times higher than that of Cd, although the latter was more than 50 times higher than that of Pb in the liver tissue. Therefore, Cd was more readily accumulated in the liver than Pb.

The present study indicated that Cd and/or Pb exposure was associated with histopathological and ultrastructural damage in the liver, which was in accordance with similar observations that were reported in various experimental investigations on animals [29–33]. These results indicated that liver damage was caused following Cd and/or Pb exposure. In the present study, hyperemia was present in the liver of the animals following treatment with Cd and/or Pb. This led to disorganization of hepatic cords, degeneration of specific hepatocytes, necrosis, and microcytic steatosis. Following co-treatment with Cd and Pb, the cell damage was increased and the hepatic cord structure disappeared compared with that of the single treatment group. Several hepatocytes exhibited increased number of vacuoles and nuclear condensation. Moreover, certain nuclei were ruptured and disappeared. We further examined additional changes in the ultrastructure of the cells, which were associated with mitochondrial swelling, membrane structure destruction, and cell deformation, as well

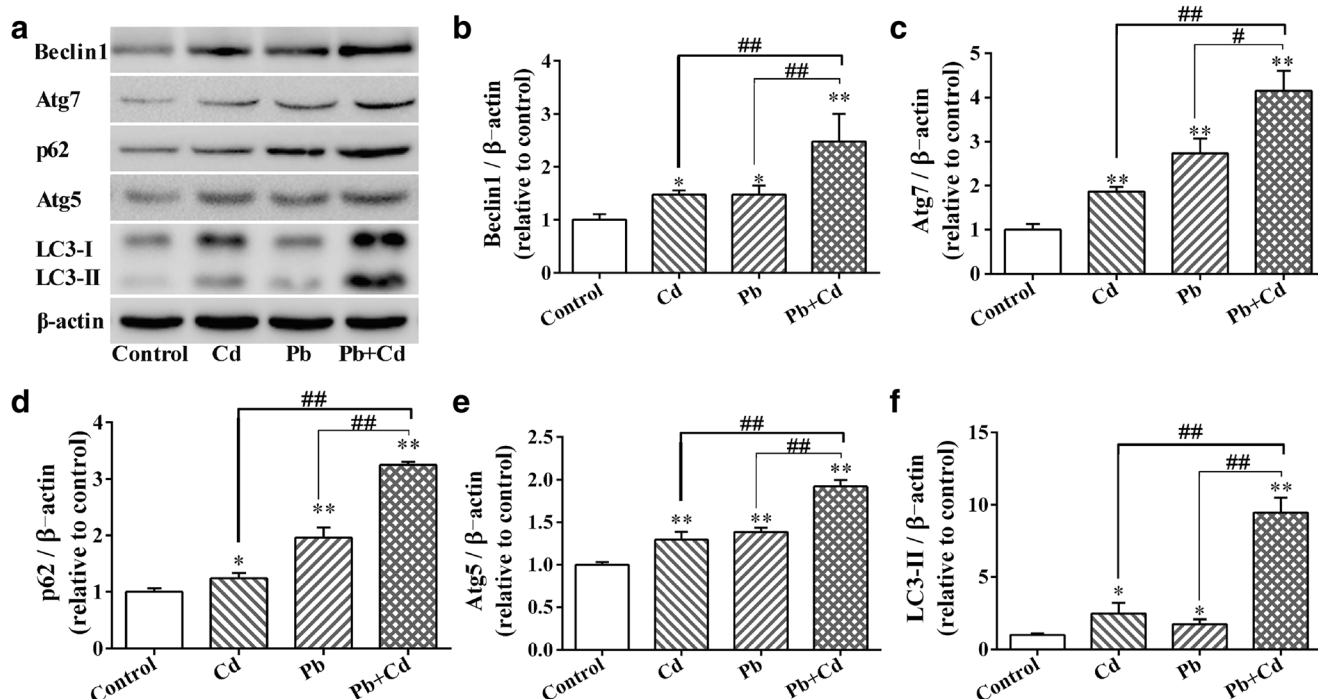


Fig. 4 Effects of Cd and/or Pb exposure on autophagy-associated proteins in the liver tissue. The protein expression levels of LC3, p62, Atg5, Beclin1, and Atg7 (b, c, d, e, f). The results were shown as mean \pm SD of

signal intensities from three independent experiments, normalized to the signal in the Control group which was set as 1. * $P < 0.05$, ** $P < 0.01$; # $P < 0.05$, ## $P < 0.01$

as chromatin concentration and marginalization. These changes were noted in the treatment group compared with the control group. The combined treatment group exhibited aggravated ultrastructural damage in the liver with increased number of vacuoles in the cells, reduced cytoplasm, and nuclear disappearance.

Oxidative stress refers to the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells following stimulation by the internal and external environment, which exceeds the antioxidant scavenging capacity of the body and disrupts the balance between oxidation and antioxidant defense. This causes the accumulation of oxygen-free radicals in the body, promoting damage to the body tissues and cells [34]. The induction of oxidative stress is a major part of the mechanism of Cd and Pb toxicity. The influence of Cd and Pb on the redox system of the body is also associated with the concentration and duration of Cd and/or Pb exposure [35, 36]. A previous study has shown that Pb can bind to functional SH-groups and inhibit the activity of SH-containing molecules. Therefore, the antioxidant system is destroyed and oxidative stress is induced [37]. Flora et al. reported that following exposure to high concentration of Pb, the activity of the antioxidant enzymes was upregulated, which may be the response of the body to the increase of reactive oxygen species [8]. The influence of Cd exposure on the redox system of the body is similar to that of Pb. Cd can competitively inhibit Fe, Cu, Zn, and other bivalent metal ions, resulting in inhibition of antioxidant enzyme activity [38,

39]. Cd can further stimulate the activity of the antioxidant enzymes in the body in order to reverse cell damage [40]. In the present study, the animals were treated with Pb or Cd for 12 weeks (50 mg/l Cd, or 300 mg/l Pb). These chemicals were included in their drinking water. The results indicated that with the exception of SOD levels, Pb or Cd treatment could upregulate CAT and GSH activity levels and downregulate GSH-Px activity. The different effects caused on MDA levels may be associated with different stages of the body's resistance to oxidative stress. Moreover, we also found that cadmium could upregulate the content of Cu, Zn, and Mn, which was associated with the increase of the antioxidant enzyme activity. Cd could significantly downregulate the Fe content, which was possibly due to the fact that Cd could bind to ferric transporter proteins and competitively inhibit the transport of ions.

Autophagy is widespread in eukaryotic cells and is a programmed intracellular degradation process. It plays an important role in cell growth and division. Abnormal autophagy is closely associated with the development of tumors, neurodegenerative diseases, diabetic nephropathy, and other diseases [40]. Previous studies have shown that the introduction of heavy metals into the body can induce autophagy in the cells [41, 42]. However, the role of autophagy in promoting or inhibiting heavy metal poisoning is not completely clear. In retinal pigment epithelial cells, Cd exposure induced autophagy [43]. The study by Huang et al. indicated that Pb could significantly upregulate the expression levels of autophagy-

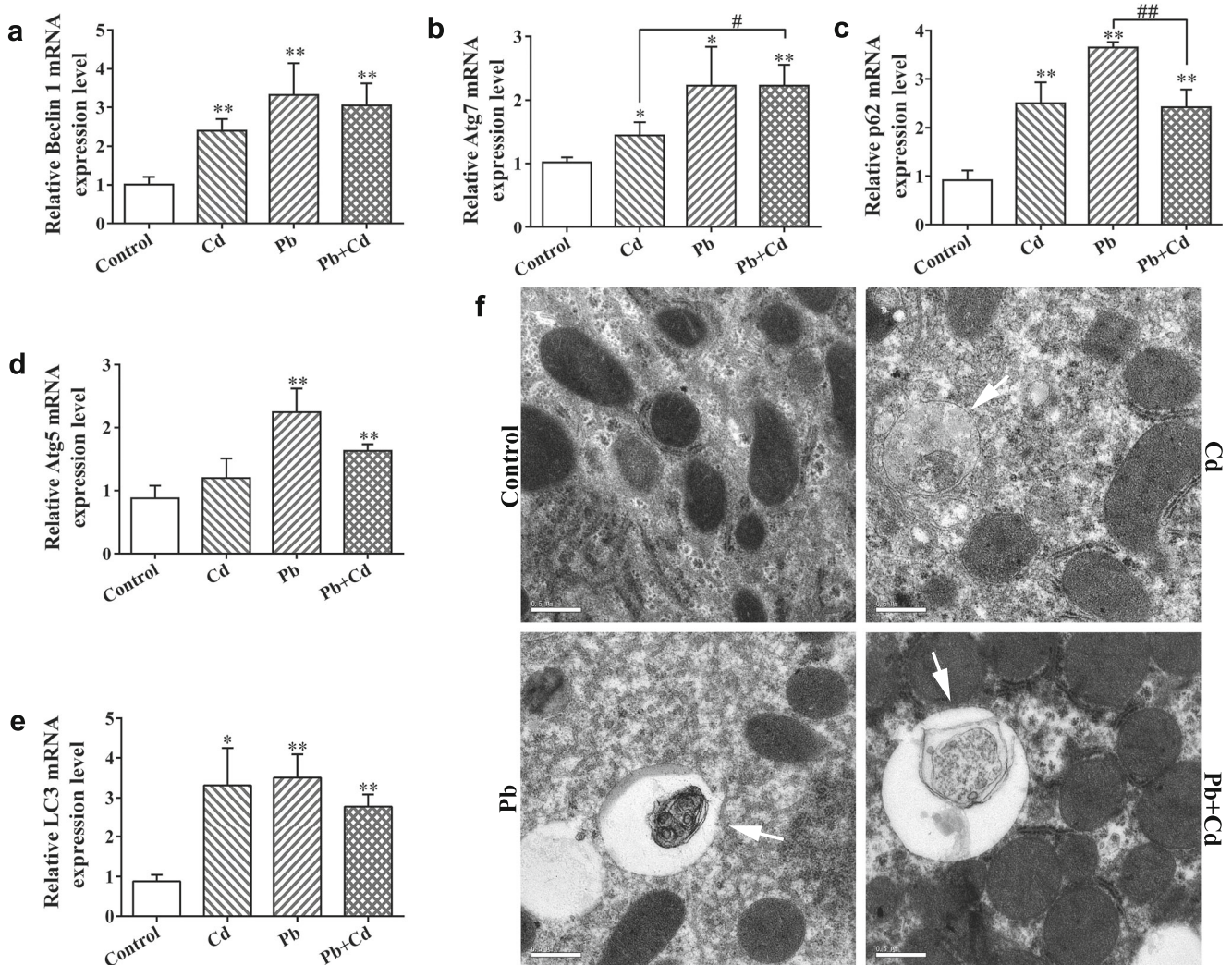


Fig. 5 Effects of Pb or Cd on autophagy-associated genes and autophagosomes in the liver tissue *Beclin1* (a), *Atg7* (b), *P62* (c), *Atg5* (d), and *LC3* (e) gene transcription levels were detected by RT-PCR. Ultrastructural modifications in the mitochondrion of mouse Sertoli cells

following inhalation of Pb, Cd or Pb-Cd mixture. (f) TEM was used to detect the formation of autophagosomes. * $P < 0.05$, ** $P < 0.01$; # $P < 0.05$, ## $P < 0.01$

associated proteins, such as Atg5, Beclin-1, and LC3, which in turn caused activation of protective autophagy [44]. The results of this experiment indicated that both Cd and Pb could significantly upregulate the expression levels of autophagy-associated proteins, such as Atg7, Atg5, Beclin-1, P62, and LC3 and the transcription levels of their corresponding genes. In the case of Cd + Pb co-exposure there was an increase of p62 protein expression. However, the mRNA levels of this group were significantly lower. This indicates a possible slowdown of p62 autophagic degradation.

The cytotoxicity caused by single treatment of Cd or Pb has been extensively studied, whereas the toxicity caused by Cd and Pb poisoning has not been fully characterized. The cognition of the change of toxicity effect of Cd and Pb is not unified. Bizarro et al. used an atomization inhalation poisoning method and examined the ultrastructural damage caused on testicular supporting cells by Pb and Cd in testicular tissues

from mice [45]. In the present study, the liver tissues of the rats were obtained following poisoning, and the histopathological and ultrastructural changes were observed. The results indicated that the exposure of the animals to Cd or Pb caused damage to the liver tissues and destroyed the structure of organelles, whereas the combined treatment of Cd and Pb aggravated the cell damage caused by single treatment of either of these metals. Wang et al. demonstrated that the combination treatment of Cd and Pb exhibited an apparent interaction on the redox system of the cells compared with that noted following single treatment of the animals with either of these chemicals [46]. Dai et al. induced a subchronic Cd and Pb poisoning to young SD rats by gavage and demonstrated that Cd and Pb poisoning exhibited a synergistic effect on oxidative damage of liver and kidney tissues in rats [47]. In the present study, the MDA levels in the lead-cadmium combination group were decreased significantly, indicating that the

lead–cadmium combination treatment promoted the activation of the antioxidant system and accelerated the removal of this oxidative stress marker. The changes noted following the combination treatment of the animals with Cd and Pb were consistent with those observed in the single cadmium treatment group indicating that cadmium played a leading influence on the redox system. Previous studies have shown that both Cd and Pb can promote autophagy. Therefore, we further tested the expression of autophagy-associated proteins following the combination of Cd and Pb; the results suggested that the combined treatment of the animals with Cd and Pb increased the expression levels of these proteins and may increase the levels of autophagy.

In conclusion, Cd and Pb caused damage to liver cells in rats by activating the body's antioxidant system and inducing autophagy. The combined treatment of Cd with Pb exacerbated this change. However, the role of the disorder of the redox system and the high levels of autophagy caused by Cd- and Pb-induced cell damage require further investigation.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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