Journal of Inorganic Biochemistry 190 (2019) 67–74

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Methylmercury-induced testis damage is associated with activation of oxidative stress and germ cell autophagy

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ARTICLE INFO

Keywords:
Methylmercury
Germ cells
Autophagy
Apoptosis
Oxidative stress

ABSTRACT

Methylmercury (MeHg) is a widespread environmental pollutant and causes a serious hazard to testicular development and spermatogenesis. However, molecular mechanisms underlying male reproductive toxicity induced by MeHg remain elusive. The objective of this study was to explore the effects of MeHg on autophagy induction in germ cells (GCs). In this study, we showed that orally administered MeHg 10 mg/kg per day for five consecutive days resulted in reduced sperm count and impaired sperm motility. Noteworthy, MeHg impaired the seminiferous tubule of rats and increased the apoptotic index of GCs of rats. Furthermore, the levels of the autophagy markers light chain 3-II (LC3-II) and beclin-1 were significantly increased following MeHg treatment, possibly via inhibiting the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (m-TOR) signaling pathway. In addition, these effects are concomitant with the overgeneration of reactive oxygen species (ROS) and the decreased expression of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Interestingly, supplementation with MeHg induced oxidative DNA damage in testes of rats. Taken together, our data indicated that MeHg stimulates GC apoptosis through oxidative stress and autophagy, which may be the mechanism responsible for the regulation of testis function and differentiation following MeHg exposure.

1. Introduction

Methylmercury (MeHg) is a global, highly toxic environmental pollutant [1]. The primary environmental route of MeHg exposure is from dietary sources to aquatic systems [2,3]. The human health risks associated with MeHg exposure are associated with poisoning; for example exposure to contaminated grain in Iraq and contaminated fish in Japan led to developmental retardation, neurological abnormalities, motor damage, and ataxia [4,5].

MeHg is absorbed in the stomach and intestine and is readily distributed to various tissues in the body. Previous work has demonstrated that MeHg induces oxidative stress through increasing intracellular reactive oxygen species (ROS), which plays an important role in MeHg-induced neurotoxicity [6]. Such MeHg-induced ROS generation and protein modification may lead to abnormal accumulation of impaired proteins. Autophagy is a highly evolutionarily conserved cellular process in eukaryotes for degrading long-lived or unfolded proteins, impaired organelles, ROS and recycling amino acids [7]. Autophagy is characterized by the formation of double-membrane structures (autophagosomes), engulfing cytoplasmic material and subsequently fusing with lysosomes for degradation [8]. Recent studies have demonstrated the occurrence of autophagy following mercury exposure [9,10]. In addition, MeHg induces autophagy in rat primary astrocytes and human neural stem cells, resulting in apoptosis of human neural stem cells and death of human hepatic cells [11,12]. These observations indicate that autophagy may play an important role in MeHg toxicity.

Methylmercury has been shown to affect male reproductive function. Mercury inhibits the activity of Leydig cell enzymes involved in steroidogenesis and membrane function, resulting in impaired production of sex steroids, such as testosterone [13–15]. Furthermore, impairment of the production of testosterone can disturb male reproduction by impairing spermatogenesis, among other effects [16]. Previous research has shown that exposure to mercury led to a significant decline in the number of spermatogonia, spermatocytes and spermatids in testes of rats [17]. Shino Homma-Takeda et al. have shown that MeHg impairs male rat spermatogenesis, resulting in germ cell deletion via cell- and stage-specific apoptosis [18]. However, the molecular mechanism of MeHg on the apoptosis of spermatogenesis

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https://doi.org/10.1016/j.jinorgbio.2018.10.007
Received 11 July 2018; Received in revised form 15 October 2018; Accepted 16 October 2018
Available online 18 October 2018
0162-0134/

remains unclear. Given the impact of MeHg as a common pollutant, finding a relationship between MeHg toxicity and autophagy would shed new light on the role of autophagy in stress responses. In the present study, we established rat models to determine the effect of MeHg on the activity of autophagy and oxidative stress in GCs and to explore its underlying molecular mechanism in the mammalian target of rapamycin (mTOR) signaling pathway.

2. Materials and methods

2.1. Chemicals

Methylmercury chloride was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The dihydroethidium (DHE) fluorescence probe (Vigorous Biotechnology Beijing Co, Ltd., Beijing, China) was used to measure ROS levels. The kits used to test the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and solvents were of analytical grade.

2.2. Animals and treatments

Nine- to ten-week-old male Sprague-Dawley (SD) rats (280–300 g) were obtained from the animal center of Huazhong University of Science and Technology. The rats were housed with a 12 h light/dark cycle under controlled temperature conditions (23 ± 3 °C), and were fed purified water and standard diet. After 1 week of acclimatization, rats were randomly divided into four groups of six rats each: three MeHg treatment groups and a control group. The MeHg-treated groups of rats were orally administered MeHg (10 mg/kg, body weight, dissolved in 5 mM Na_2CO_3) for 5 days. Control rats were orally administrated with equal volumes of distilled water. The mode of administration was chosen based on a previous study [19]. All rats were sacrificed by decapitation on 12, 19, and 26 days after the first MeHg administration. The testes and epididymis were collected immediately. The cauda epididymides were used to measure sperm parameters (sperm count and sperm motility). The dissected tissue of each left testis was used for histopathological analysis, immunohistochemistry and detection of GC apoptosis by Terminal deoxynucleotide transferase mediated dUTP-biotin nick end labeling (TUNEL), and the dissected tissue of each right testis was used for electron microscopy observation and ROS evaluation. The remaining tissues were stored at −80 °C for mercury concentration measurement, biochemical and western blotting analysis. All experiments were done in the isolation cabinet. In addition, the researchers all wore goggles, thickened masks and gloves in carrying out the experiments.

2.3. Measurement of mercury concentration in testis

Total mercury concentrations in testes of rats were evaluated by oxygen combustion–gold amalgamation method. Samples of the testes were homogenized in distilled water (10% weight/volume). The level of mercury in testis were determined by atomic absorption spectroscopy according to the method describe by D.H. Nam et al. [20].

2.4. Sperm motility and sperm count

Spermatozoa were obtained immediately from the left cauda epididymis of rats after sacrifice and were immediately diluted with 1.5 mL of physiological saline, pre-warmed to 37 °C. Then, a 20 uL aliquot was transferred to a glass slide at 37 °C. Total sperm and sperm motility were counted by using a hemocytometer under a light microscope at 200× magnification. The spermatozoa were classified as progressive, nonprogressive and immotile. Sperm motility was expressed as % total sperm.

2.5. Histology

For histopathological examinations, the testes were fixed in 10% buffered formalin for 24 h, and embedded in paraffin after dehydration in graded ethanol. Paraffin blocks were cut into 5-μm-thick sections, rehydrated with gradient ethanol, and stained with hematoxylin-eosin (H&E).

2.6. Terminal deoxynucleotide transferase mediated dUTP-biotin nick end labeling (TUNEL)

Testicular GC apoptosis was assessed by TUNEL assay using an In Situ Cell Death Detection, POD Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The dewaxed tissue sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP, for 60 min at 37 °C. The slides were then washed three times with phosphate buffer saline (PBS) and incubated with the secondary anti-fluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine was added to sections for chromogenic reaction. The sections were then counterstained with hematoxylin. The stained sections were observed at 400× magnification. The positive cells were stained brown. The apoptotic index (AI) was calculated as follows: AI = (number of apoptotic cells per section/total number of cells per section) × 100%.

2.7. Evaluation of oxidative stress and oxidative DNA damage

CAT, GPx and SOD analysis was performed on testicular tissue homogenate. CAT activity was detected by measuring the decomposition of hydrogen peroxide (H_2O_2) as an increase in absorption at 540 nm for 10 min, according to the spectrophotometric method [21]. One unit of CAT activity was defined as the consumption of 1 μmol H_2O_2 per min. GPx activity was assessed by using a glutathione reductase assay with 5 mM glutathione and 0.25 mM H_2O_2 as substrates at 25 °C [22]. A unit of GPx is defined as the consumption of 1 mmol of nicotinamide adenine dinucleotide phosphate (NADPH) per min. SOD activity was measured according to the method describe by Kakkar et al. [23], and a unit of SOD activity was defined as the enzyme concentration required to decrease the chromogen production by 50% per min. ROS were detected in the testes with DHE in situ. Testis sections were embedded in an optimum cutting temperature compound, frozen, and then cut into 4-μm-thick sections. Following incubation with 10 μM DHE at 37 °C for 30 min in the dark, the sections were examined using a fluorescence microscope (Leica Imaging Systems Ltd., Cambridge, UK), and images were captured at 400×. Red fluorescence represents superoxide production. The intensity of the fluorescent signal was quantitated by Image J software. The oxidative DNA damage in the rat testes after administration of MeHg to rats were investigated by measuring 8-hydroxydeoxyguanosine (8-OH-dG), an oxidative DNA product. Testis DNA was immediately isolated as the method described before [24]. The isolated DNA was stocked in dried state under argon at 4 °C and digested to deoxynucleotides with nuclease PI and alkaline phosphatase. The levels of 8-OH-dG were analyzed by highperformance liquid chromatography-electrochemical detection system (Chelmsford, MA, USA).

2.8. Western blotting

Total protein was immediately extracted from testicular tissue, and the protein content was quantified by the bicinchoninic acid protein assay kit (Applygen Technologies Inc., Beijing, China). Proteins from each group were separated using SDS-polyacrylamide gel electrophoresis (PAGE), followed by transfer to nitrocellulose membranes. Membranes were incubated with a blocking solution for 1 h, and then incubated with the indicated antibodies at 4 °C overnight. Membranes were incubated with the appropriate horseradish peroxidase-

conjugated secondary antibodies for 1 h at 37 °C. The protein bands were visualized by enhanced chemiluminescence (ECL) Plus western blotting detection system. Anti-beclin-1, light chain 3 (LC3), phosphoinositide 3-kinase (PI3K), phosphorylated-protein kinase B (p-Akt), protein kinase B (Akt), phosphorylated-mammalian target of rapamycin (p-mTOR), mTOR, antibodies were purchased from Sigma Chemical Co. (St. Louis, MO, USA). An anti-β-actin antibody was purchased from Boster Bio-Technology Co. (Wuhan Boster Bio-Technology Co., Ltd., China).

2.9. Transmission electron microscopy (TEM)

Testicular tissues from the control group and the MeHg-treated groups were fixed with 2.5% glutaraldehyde and 1.4% sucrose in 0.1 M cacodylate buffer (pH 7.4, 37 °C for 1.5 h, post-fixed in 1% osmium tetroxide, and embedded in Epon-812. The ultra-thin sections were cut into semi-thin using a diamond knife, and stained with methanolic uranyl acetate and lead citrate. The testicular ultrastructure images were obtained using TEM (JEM 1200-EX; Hitachi, Ltd., Tokyo, Japan).

2.10. Statistical analysis

The data are expressed as the mean ± S.D. Differences among groups were analyzed by one-way analysis of variance (ANOVA). Fisher's least significant difference test was used to determine statistical significance ($p < 0.05$).

3. Results

3.1. Analysis of mercury concentration in testis

Mercury concentration was performed to assess the absorption of Mercury in testis (Fig. 1). In the MeHg-treated group, the levels of mercury in testis mercury continued to increase in 12 days group and 19 days group. In line with previous study [19], the delayed peak of mercury concentration may be close relation to the slow uptake rate of mercury by in testis.

3.2. MeHg reduces sperm motility and sperm count

In MeHg-treated groups, sperm quantity was decreased dramatically ($p < 0.01$) at 26 days after the first administration of MeHg compared with the control group (Fig. 2 A). In addition, the levels of Type A spermatozoa were decreased significantly in the MeHg-treated rats as compared with the control; this was accompanied by increases in both Type B and Type C sperm (Fig. 2 B).

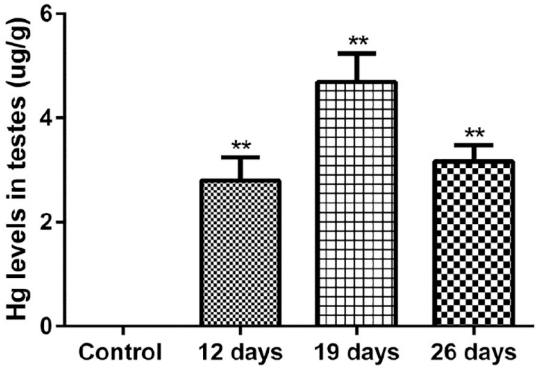

Fig. 1. Mercury levels in testis of rat
Testis mercury concentration continued to increase until 19 days after the first MeHg administration. Values are expressed as medians. All data are expressed as the mean ± S.D. ($n = 6$). Compared with the control ** $p < 0.01$.

3.3. Histological evaluation

Histological evaluation was performed to assess the damage effect of MeHg on testes using H&E staining (Fig. 3), revealing that rats in the control group had normal histological features of seminiferous tubules. No obvious damage was found at 12 days or 19 days after the first treatment with MeHg. However, the seminiferous tubules exhibited a significant disruption of the germinal epithelium, revealing few spermatozoa at 26 days after the first administration of MeHg.

3.4. Effects of MeHg on apoptosis of GCs

TUNEL analysis was performed to investigate whether MeHg was able to induce GC apoptosis in the testis (Fig. 4). Notably, MeHg was found to influence the apoptosis of GCs. In the 19 and 26 day groups, the apoptosis of GCs was decreased dramatically ($p < 0.01$) compared with the control group. No significant differences were detected in the 12 day group ($p > 0.05$).

3.5. MeHg induced oxidative stress and oxidative DNA damage in the rat testis

We measured the level of antioxidant enzymes including CAT, GPx and SOD. The results were found to be in line with the ROS and TUNEL results. MeHg exposure significantly decreased the CAT, GPx and SOD activities (Fig. 5 A). To investigate the role of ROS in MeHg-induced GC apoptosis, the level of ROS was measured. After exposure of rats to MeHg, the level of ROS significantly increased (Fig. 5 B—C). To further confirm the role of oxidative stress in modulating apoptosis induced by MeHg, the oxidative DNA damage in the testis after administration of MeHg to rats were evaluated by measuring 8-OH-dG, an oxidative DNA product. The treatment of MeHg increased the 8- OH-dG level in testis (Fig. 5 D). These results suggest that oxidative stress may be involved in MeHg-induced apoptosis in testis.

3.6. MeHg promotes GC autophagy in the rat testis

To verify the induction of GC autophagy in the rat testis after MeHg treatment, transmission electron microscopy (TEM), the gold standard for determining autophagy, was performed. As shown in Fig. 6 A, GCs in rats treated with MeHg (12 day group, 19 day group and 26 day group) had an obvious increase in the number of autophagosomes and autolysosomes compared with the control group, suggesting that autophagy induction may be related to the apoptosis of GCs. Beclin-1 and LC3 have been suggested to be very important marker proteins of autophagy [25,26]. We next investigated the expression of these two proteins. Interestingly, treatment with MeHg led to a remarkable increase in the protein expression of beclin-1, as well as LC3-II compared with the control group (Fig. 6 B). Altogether, these results strongly support that MeHg is capable of inducing GC autophagy in the testes of adult male rats.

3.7. MeHg-induced autophagy is related to the inhibition of the phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway

The PI3K/Akt/mTOR signaling pathway has been suggested to be one of the most important regulators of autophagy [27,28]. We further investigated whether MeHg induces autophagy through regulation of

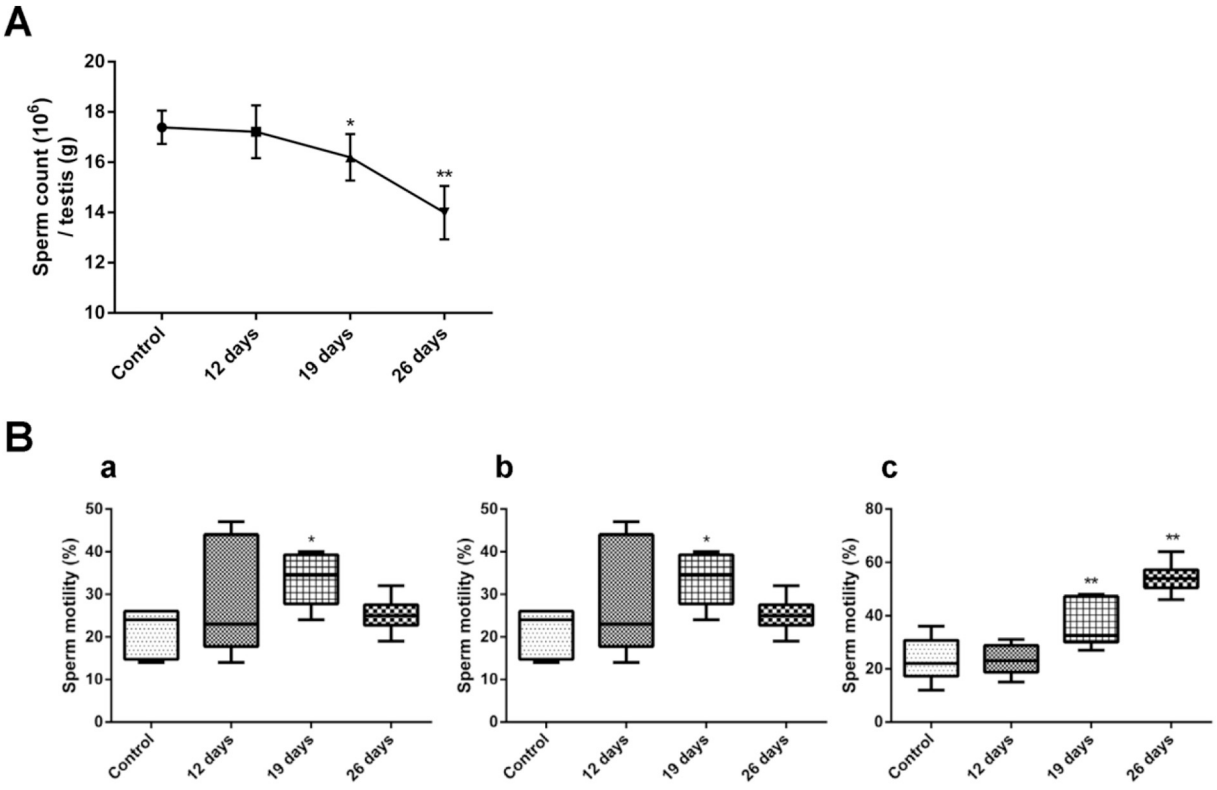

Fig. 2. Effects of methylmercury on sperm count and sperm motility
Sperm count (A) and sperm motility (B). Sperm motility Type A: mobile with progression (B-a), Type B: mobile without progression (B-b) and Type C: immobile (B-c). Values are expressed as medians. All data are expressed as the mean ± S.D. ($n = 6$). Compared with the control * $p < 0.05$, ** $p < 0.01$.

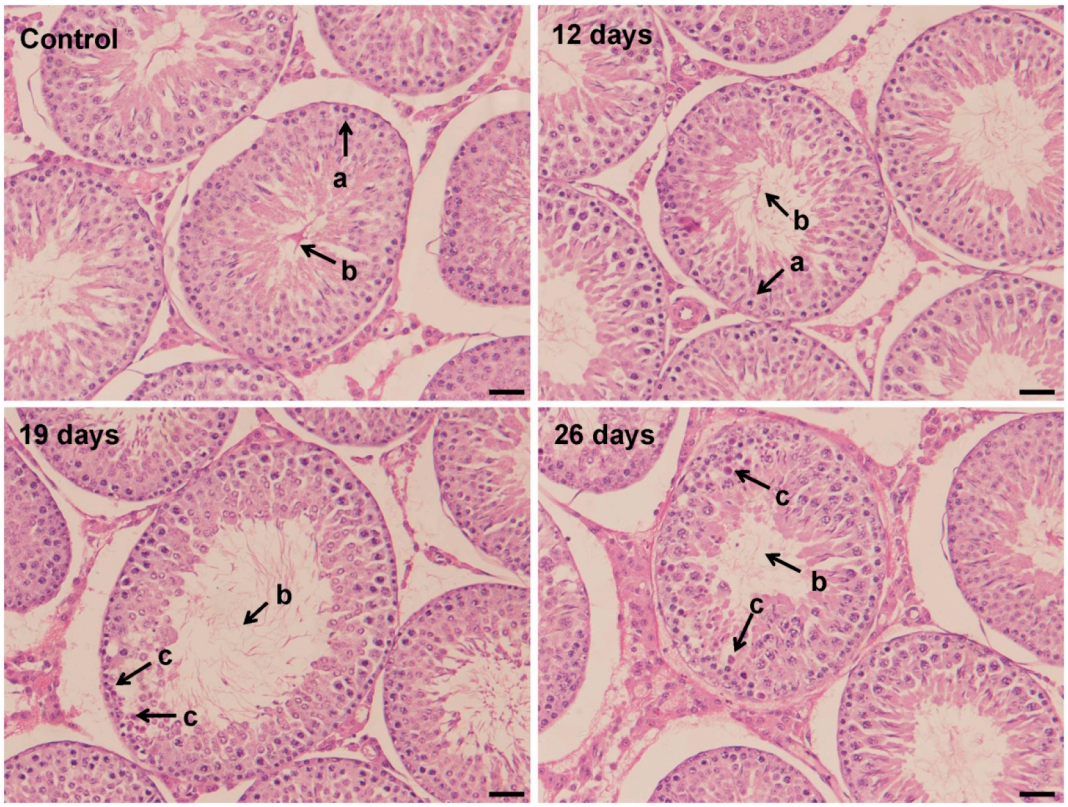

Fig. 3. Methylmercury exposure impaired testicular damage as assessed by histopathology
The testis of the control showed a normal structure with normal spermatogenic cells (a) and spermatozoa (b). There are no obvious testis histopathological abnormalities at the light microscope level 12 days after the first administration. MeHg-treated groups at 19 days and 26 days after the first administration showed abnormal germ cells (c) and few spermatozoa (b). Original magnification: 200×. Scale bar is 50 μm.

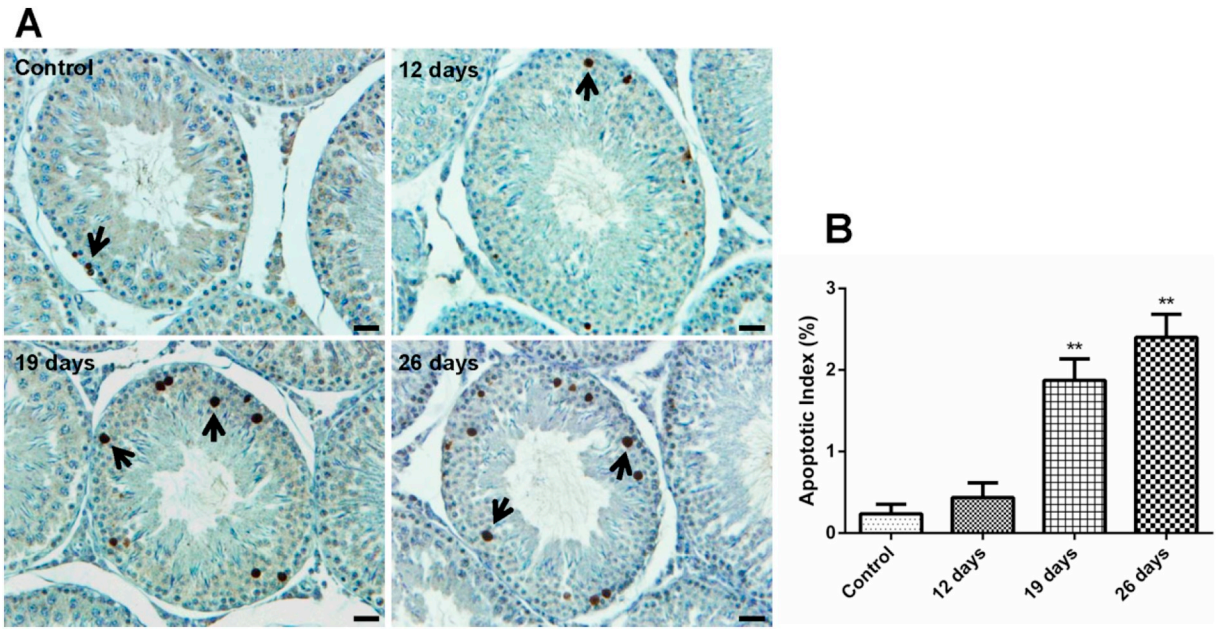

Fig. 4. Methylmercury-induced GC apoptosis
(A) Representative photomicrographs of a TUNEL-stained testis section from the control group (Control) and MeHg-treated groups 12, 19 and 26 days after the first administration. Arrows indicate TUNEL-positive apoptotic cells in the testis. (B) Apoptotic index. All data are expressed as the mean ± S.D. (n = 6). Compared with the control * $p < 0.05$, ** $p < 0.01$. Original magnification: 400×. Scale bar is 50 μm.

the PI3K/Akt/mTOR pathway in testis. As shown in Fig. 7, the level of PI3K was up-regulated after MeHg administration. Meanwhile, compared with the control group, the expression of phosphorylated-AKT and phosphorylated-mTOR were significantly decreased at 19 days and 26 days after the first treatment with MeHg. Together, these results suggest that the PI3K/Akt/mTOR signaling pathway is involved in MeHg-induced autophagy in the rat testis.

4. Discussion

There are three forms of mercury (Hg): organic, inorganic and elemental. Each form has its own profile of toxicity. The most general forms of Hg in the environment are metallic Hg, including methylmercury, inorganic salts, mercuric chloride, and mercuric sulfide. MeHg has long been a focus for concern due to its effects on human and ecological health through bioaccumulation via the food chain [29]. For example, MeHg treatment can activate mitochondrial-dependent caspase-3 in the brains of rats, accompanied by an obvious decrease in hippocampal cell viability and an increase in cell apoptosis [30]. Even a low dose of Hg can cause the induction of cell death in vitro. In addition, MeHg exposure resulted in mitochondrial dysfunction and T-cell apoptosis, and at the same time induces death signaling pathways [31]. Noteworthy is that the present study revealed that the treatment of MeHg robustly induced the apoptosis of GCs, and simultaneously impaired the quality of sperm in rats, which is consistent with previous studies [32,33]. Normal spermatogenesis is highly dependent on natural differentiation and development of GCs; conversely, GC dysfunction leads to oligoasthenospermia, or even infertility. After administration of MeHg, significant decreases in testosterone level, sperm motility and sperm quantity were found [32,34]. Moreover, S Homma-Takeda et al. reported that MeHg impaired spermatogenesis by stage- and cell-specific GC apoptosis in rats [18]. These studies indicate that autophagy plays an important role in MeHg-induced male reproductive toxicity.

A plethora of studies have illustrated that oxidative stress acts as a master mechanism of MeHg toxicity [35–37]. Oxidative stress is a multifaceted process and is known to act either as pro-survival or pro-death signaling. The mechanisms of MeHg-induced oxidative stress involve the excessive accumulation of ROS and the inhibition of antioxidant enzymes [38–41]. However, a detailed investigation of the role of oxidative stress in MeHg reproductive toxicity remains unknown. In this study, we have demonstrated that MeHg is able to inhibit the activity of the antioxidant enzymes, including CAT, GPx and SOD, which are positively related to sperm motility [42,43]. On the other hand, our results show that MeHg induced overproduction of ROS in the testis of rats. In addition, we demonstrated that MeHg induced oxidative DNA damage in rat testis following oral exposure of MeHg. The cell apoptosis is attributed to persistent production of ROS and DNA damage [44,45]. The concordance analysis of this study showed strong correlations between oxidative DNA damage and GC apoptosis in the testis, indicating that oxidative stress may play an important role in the MeHg-induced GC apoptosis. Apoptosis and autophagy are defined as programmed cell death [46]). Both may cooperate and lead to cell death during this process [47]. Oxidative stress as an important mediator in autophagy and apoptosis has been well identified [48]. There are substantial studies indicating that oxidative stress participates in autophagy to regulate cell apoptosis [49,50]. Interestingly, oxidative stress can also induce apoptosis and lead to impairments of semen quality and male fertility [51,52]. Yet whether MeHg can induce GC autophagy in testes and play a cytotoxic role in testis function remains elusive.

Autophagy is one of the master pathways responsible for eliminating misfolded, aggregated, or damaged proteins and organelles. Microtubule associated protein LC3 is a well-established marker of autophagy. During autophagy, LC3 is important in the formation of autophagosomes and is also essential for the development of autophagy [53]. Moreover, the ratio of LC3-II/LC3-I is a consistent marker for the detection of autophagy [54]. In addition, the autophagic proteins beclin-1, autophagy-related 5 (Atg5) and autophagy-related 7 (Atg7) are

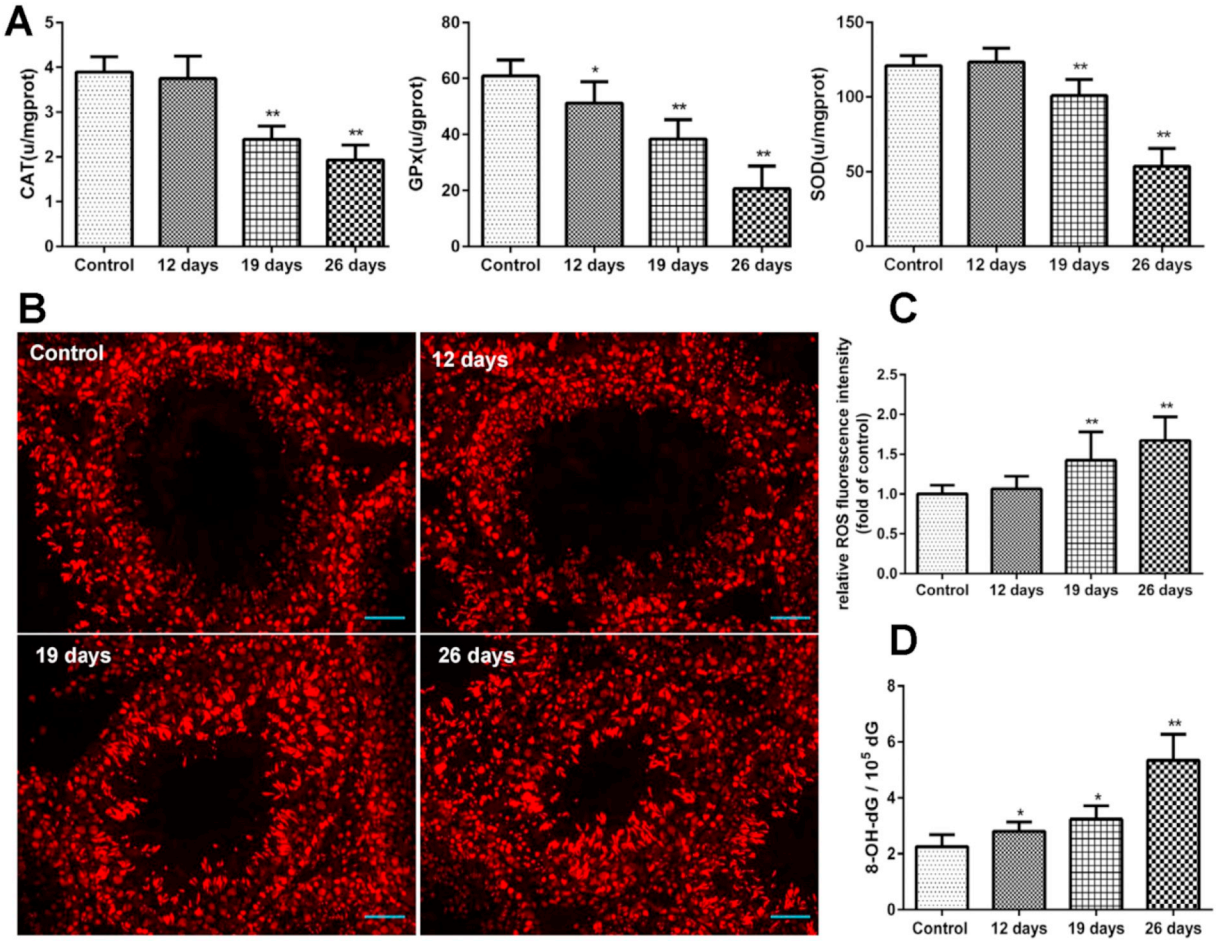

Fig. 5. Methylmercury-induced oxidative stress and oxidative DNA damage in testis
(A) MeHg inhibited the activity of antioxidant enzymes. (B) ROS levels were increased in the MeHg-treated group as compared with the control group. Red fluorescence represented the overall testicular oxidation levels. (C) Relative ROS fluorescence intensity. (D) Effect of MeHg exposure on the 8-OH-dG level in the testes of rats. All data are expressed as the mean ± S.D. (n = 6). Compared with the control * $p < 0.05$, ** $p < 0.01$. Original magnification: 400×. Scale bar is 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

responsible for autophagosome biogenesis. Beclin-1 is required for autophagic gene Atg5/Atg7-dependent or independent autophagy [55]. In the present study, we found a clearly elevated level of LC3-II induced by excessive MeHg administration, as well as by an elevation in the beclin-1 protein, indicating the induction of autophagy in testis. In order to further confirm it, we checked the ultrastructural details of autophagosomes using TEM, the gold standard for determination of autophagy. Rats treated with MeHg, even at 12 days after the first administration, had a distinct increase in the number of autophagosomes and autolysosomes in GCs, but no obvious histopathological damage was found. We consider that autophagy, as an intracellular protein degradation process in GCs, might be very sensitive to toxicants such as MeHg. Altogether, these results strongly support that MeHg is capable of inducing GC autophagy in the testes of male rats.

The mTOR kinase is critical in regulating autophagy is modulated by oxidative stress, nutrient depletion, or low energy [56]. The mTOR is activated in the presence of abundant cellular nutrients, and functions as a depressor of the initiation step of autophagy. In addition, mTOR colocalizes with LC3, participating in the formation of autophagosomes [57] Moreover, inhibition of mTOR leads to the induction of autophagy [58]. Processing of the pre-autophagosomal structure requires the beclin-1–PI3Kcomplex, as well as the insertion of LC3-II into the autophagosomal membrane [59]. The PI3K/Akt/mTOR pathway is a central signaling pathway involved in autophagy. Previous studies have demonstrated that the PI3K/Akt/mTOR signaling pathway is responsible for autophagy induction [60]. Chien-Ju Lin et al. reported that honokiol induced significant autophagic cell death via regulation of the PI3K/Akt/mTOR signaling pathway [61]. Accumulating evidence indicates that autophagy plays an important role in alcohol-induced liver pathophysiology. Interestingly, the PI3K/Akt/mTOR signaling pathway plays a critical role in alcohol-induced autophagy in liver disease [62]. Similarly, the present study revealed that autophagy was induced after treating rats with MeHg, accompanied by decreased protein levels of PI3K, p-Akt, and p-mTOR. These results confirm that the PI3K/AKT/mTOR signaling pathway may be involved in MeHg-induced autophagy in GCs of rat testes.

The present study showed that MeHg can cause sperm quality disorder and oxidative stress in rat testes. Our study provides strong evidence that MeHg stimulates autophagy and inhibits the activation of the PI3K/AKT/mTOR signaling pathway in GCs apoptosis of rat testes. These results shed new light on the potential mechanisms of testicular dysfunction caused by MeHg exposure. However, the detailed role of

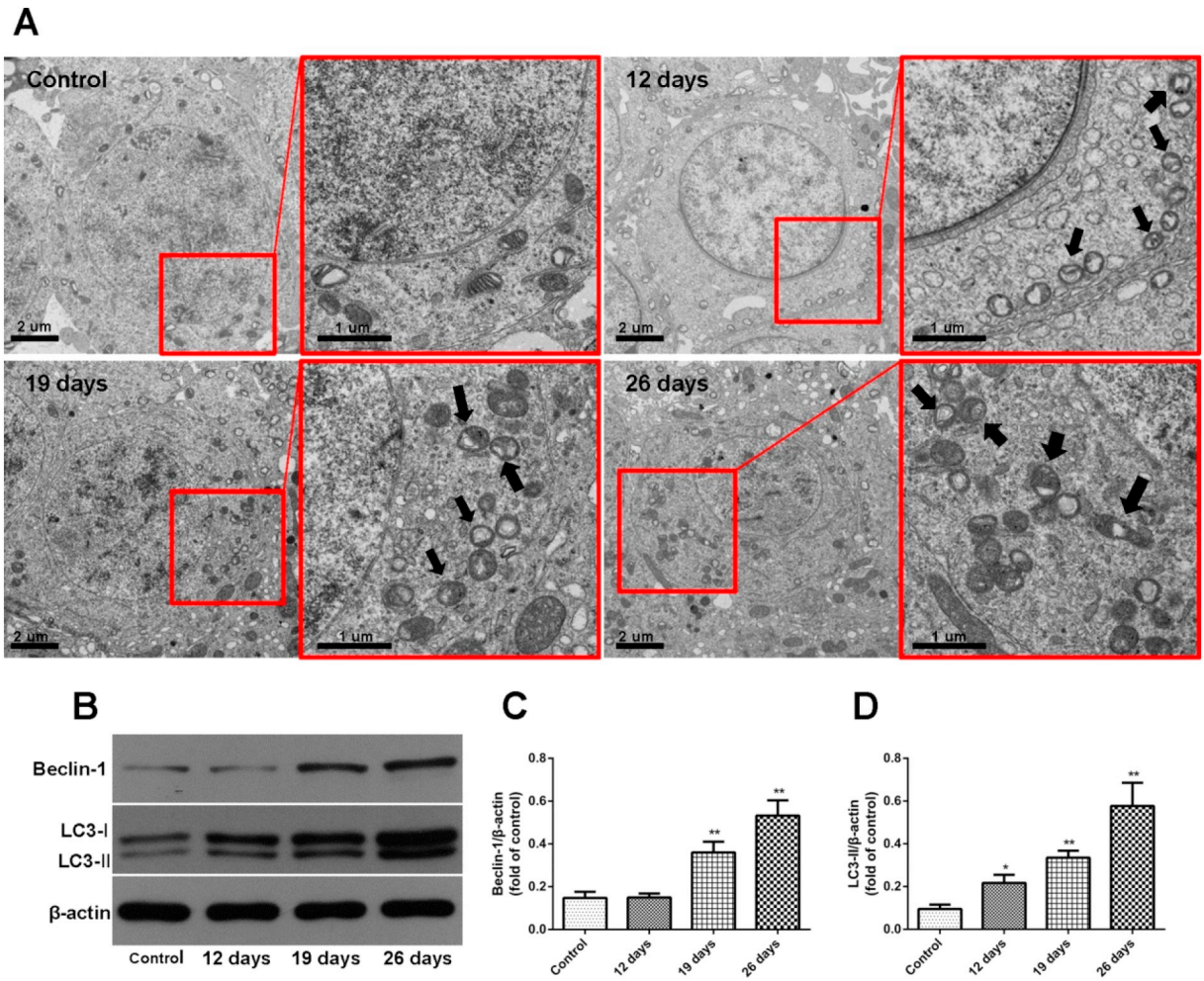

Fig. 6. Methylmercury-induced GC autophagy, which was coincidental with upregulation of autophagy-related proteins in the rat testis.
(A) Transmission electron microscopy evaluation of autophagosomes in germ cells of testis in rats. The arrows indicate formed autophagosomes and autolysosomes. (B) The effect of MeHg on the expression of autophagy markers (beclin-1 and LC3-II) in testis was analyzed by western blotting following MeHg treatment. (C) Relative expression of beclin-1. (D) Relative expression of LC3-II. All data are expressed as the mean ± S.D. (n = 6). Compared with the control * $p < 0.05$, ** $p < 0.01$. Scale bars: 2 μm and 1 μm.

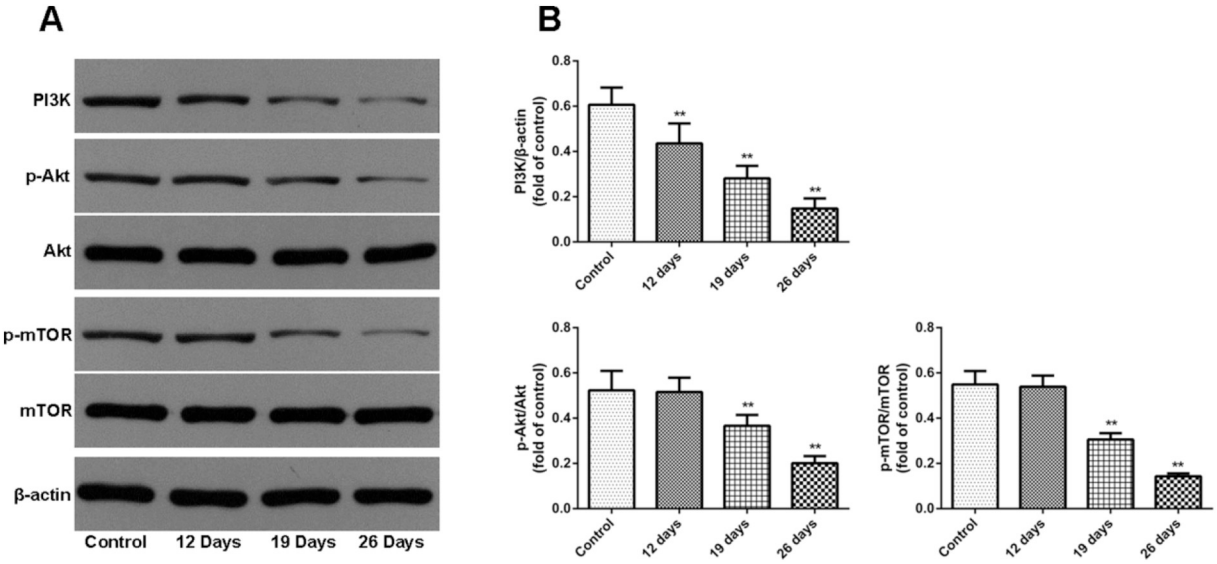

Fig. 7. Methylmercury inhibited the PI3K/Akt/mTOR signaling pathway in the rat testis
(A) Western blots showed the effects of MeHg on the expression of PI3K, p-AKT/AKT and p-mTOR/mTOR in testes. (B) Relative expression of PI3K, p-AKT and p-mTOR. All data are expressed as the mean ± S.D. (n = 6). Compared with the control * $p < 0.05$, ** $p < 0.01$.

MeHg-induced GC autophagy and the suppression of the PI3K/AKT/mTOR signaling pathway in the regulation of spermatogenesis needs to be elucidated.

Abbreviations

MeHg methylmercury
GCs germ cells
ROS reactive oxygen species
SOD superoxide dismutase
GPx glutathione peroxidase
CAT catalase
8-OH-dG 8-hydroxydeoxyguanosine
LC3 light chain 3
PI3K phosphoinositide 3-kinase
Akt Protein Kinase B
mTOR mammalian target of rapamycin
TEM transmission electron microscopy
TUNEL terminal deoxynucleotidyl transferase (TdT)-mediated deoxy-UTP nick-end labeling

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was financially supported by Natural Science Foundation of Hubei Province (No. 2017CFB117), Hubei Province health and family planning scientific research project (No. J2017Q017), Wuhan Youth Science and Technology Plan (2017050304010293), Fundamental Research Funds for the Central Universities (12060046030) and the National Science Foundation of China (No. 21705053).

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