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PM2.5 exposure induces reproductive injury through IRE1/JNK/autophagy signaling in male rats

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ABSTRACT

Fine particulate matter (PM2.5) constitutes the most significant air pollutant that causes health risks. However, the mechanism(s) underlying PM2.5-induced male reproductive injury has not been clarified. In the present study we explored whether PM2.5 activated the inositol-requiring enzyme 1 (IRE1)/c-Jun NH 2-terminal kinase (JNK)/autophagy-signaling pathway, and whether this pathway mediated reproductive injury in male rats. We established a male Sprague–Dawley rat model of PM2.5 (1.5 mg/kg) exposure-induced reproductive injury, and observed the intervention effects of STF083010 (an IRE1 inhibitor, 1 mg/kg). After 4 weeks of exposure, reproductive injury-related indicators and IRE1-cascade protein expression were analyzed. Our results showed that sperm quality and serum testosterone level significantly decreased and apoptotic index increased after exposure to PM2.5. After STF083010 intervention, sperm quality and serum testosterone level were significantly improved, while the apoptotic index was reduced. Under light microscopy, we observed that the structure of spermatogenic cells in the PM2.5 group was loose, and that the numbers of spermatogenic cells and mature spermatozoa were reduced. After STF083010 intervention, the structural damage to spermatogenic cells was improved, and the number of cells shed was reduced. Western blotting analysis showed that the expression of IRE1, phosphorylated JNK (p-JNK), beclin-1, and microtubule-associated protein 1 light chain 3(LC3)II/LC3I proteins was significantly upregulated, and that the expression of p62 protein was significantly downregulated in the PM2.5 group. The concomitant administration of STF083010 significantly antagonized the aforementioned adverse effects. STF083010 exerted specific protective effects on reproductive injury-related effects in male rats exposed to PM2.5, with effects mediated via IRE1/JNK/autophagy signaling.

1. Introduction

There has been a decline in the quality of male sperm and an increase in the incidence of infertility over the past several decades worldwide, and the reduction in the infertility rate is regarded as a major goal by the World Health Organization (Rolland et al., 2013). There are many factors that affect male reproductive functions, including environmental pollution and lifestyle (Meeker, 2010; Renu et al., 2018). In recent years, air pollution has become much more severe, and the impact of sustained smog weather on male reproductive function has been a focus of research (Cao et al., 2017; Zhang et al., 2020). Fine particular matter (PM2.5) is the most significant air pollutant that causes health risks, and it is very easy to accumulate a variety of harmful substances that enter the body by breathing; these can cause damage to multiple organ systems in the body, either directly or indirectly (Hong et al., 2016). Existing reports about the effects of PM2.5 on the body principally include actions on the respiratory system (Gao et al., 2020), cardiovascular system (Tian et al., 2019), nervous system (Huang et al., 2019), and reproductive system (Yang et al., 2019). However, the correlation between PM2.5 and reproductive injury is still undetermined, and definitive evidence on the mechanisms of male reproductive toxicity caused by PM2.5 exposure is lacking.

A recent study showed that endoplasmic reticulum (ER) stress is one of the important pathways in the survival and apoptosis of germ cells (Wang et al., 2019a). Liu et al. (2017) explored the possible underlying mechanism of PM2.5-induced reproductive toxicity in male rats in different seasons. Their results showed that expression of two ER stress proteins, i.e. glucose-regulated protein 78 kDa (GRP78) and X-box binding protein (XBP-1), were significantly increased, suggesting that reproductive toxicity mediated by PM2.5 resulted from the activation of

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ER stress. Inositol-requiring enzyme (IRE1) is one of the primary signaling pathways mediating ER stress, and IRE1 dissociates from transmembrane proteins when ER stress occurs. The activated IRE1 pathway also mediates cell survival and apoptosis. Our previous study confirmed that ER stress plays an important role in fluoride-induced testicular Sertoli cell damage; and that the expression of IRE1 was significantly increased (Yang et al., 2015). Accordingly, we assume that the ER stress molecule IRE1 may play an important role in the reproductive toxicity of PM2.5 in male rats. However, the specific mechanism by which IRE1 mediates male reproductive injury through its activation requires further investigation.

Autophagy plays an important role in maintaining cellular survival and homeostasis; and regulating many important physiologic processes such as cellular proliferation, cellular immunity, and inflammation (Paul and Münz, 2016). Under normal physiologic conditions, cells maintain a basic autophagic level, which is then increased rapidly when the cells are exposed to toxicants, nutritional energy deficiency, stress, etc. (Galati et al., 2019; Martin-Rincon et al., 2019; Wang et al., 2019b). An established PM2.5 intervention in human airway epithelial cell (HBE) and autophagy-deficient mouse models that entails autophagy inhibitors to downregulate HBE autophagy level showed that PM2.5-induced interleukin (IL)-8 expression was significantly reduced. In addition, autophagy-deficient beclin-$1^{+/-}$ mice acquired a significant protective effect against PM2.5-induced airway inflammation (Wu, 2014). Evidence has shown that autophagy is essential for ensuring the formation of specific sperm structures and the degradation of certain components during spermatogenesis (Xu et al., 2016). Autophagy also plays a key role in the regulation of the reproductive process, with a close relationship existing between autophagy and androgen-binding protein, lipid metabolism, and testosterone biosynthesis (Zhu et al., 2019). However, the role of autophagy in PM2.5-induced male reproductive injury and its mechanism remain unclear.

The c-Jun NH 2-terminal kinase (JNK) is an important member of the serine/threonine protein kinase (MAPK) family (Zeke et al., 2016). The JNK pathway is activated by a variety of extracellular signals, including ER stress, oxidative stress, ultraviolet radiation, and cytokines (Kumar et al., 2015; Yan et al., 2016). The occurrence of severe or prolonged ER stress causes overexpression of the ER stress factor IRE1; and activated IRE1 forms an IRE1-TRAF2-ASK1 complex with ASK1, thereby activating JNK (Sophonnithiprasert et al., 2017). Activated JNK is then transfers from the cytoplasm to the nucleus to regulate related downstream transcription factors, thereby exerting commensurate biologic effects (Solinas and Becattini, 2016). A recent study showed that the JNK pathway may be closely related to various forms of autophagy. For example, JNK mainly promotes the formation of autophagy by mediating beclin-1 (Zhou et al., 2015b), suggesting that IRE1 may activate JNK and play a role in the occurrence and progression of diseases by regulating autophagy. Further study is needed to verify whether PM2.5 exposure-induced male reproductive injury is related to IRE1/JNK-induced autophagy.

Since IRE1 and autophagy should not be ignored in PM2.5-induced male reproductive injury-and JNK is related to both-in this study we hypothesized that PM2.5 mediates reproductive injury in male rats by activating IRE/JNK/autophagy signaling. We established a Sprague-Dawley (SD) rat model with PM2.5 exposure provided through intratracheal instillation, and IRE1 inhibitor was given by intraperitoneal injection. After 4 weeks of exposure, we monitored body and organ weights, sperm-quality parameters, and serum testosterone level; evaluated tissue morphology, apoptosis, and other reproductive deficits; and assessed IRE1, JNK, and autophagy-related protein expression. The aim of this study was to investigate the possible mechanisms governing male reproductive injury caused by the PM2.5 exposure. A better understanding of this may provide direction toward therapeutic targets of male reproductive injury caused by PM2.5.

2. Materials and methods

2.1. PM2.5 sampling and management

We collected fine particulate matter with a large-flow sampler (YH-1000, Jingcheng, Shandong, China) in heavy-traffic sections from March 2019 to December 2019 in Zhengzhou of Henan Province, using quartz fiber membranes. The filter membranes loaded with particulate matter were cut into 1 cm × 1 cm squares and soaked in deionized-distilled water. After 3 × 30-min ultrasonic vibrations, we filtered the shaking solution through 6 layers of gauze and centrifuged it at 12,000 rpm and 4 °C for 30 min; followed by vacuum freeze-drying, concentrating, and storing the components of the suspension of fine particulate matter at −20 °C for later use. According to the experimental requirements, sterile physiologic saline was used to prepare the desired concentration within 24 h prior to instillation, and the solution was stored at 4 °C. The particular matter was shaken well and mixed thoroughly before use.

2.2. Experimental animals and treatment

A total of 40 Sprague-Dawley male rats at 4 weeks of age, weighing between 90 and 110 g, were provided by the Experimental Animal Center of Henan Province (Zhengzhou, China, License No. SCXK (Yu) 2017-0001). Animals were raised in sterilized plastic cages with corncob bedding in a controlled-environment animal room (temperature, 23 ± 1 °C; relative humidity, 50 ± 10%; photoperiod, 12 h light/dark cycle) (Feng et al., 2015). After 1 week of quarantine with no abnormalities observed, the animals were divided into 4 groups using a random number generator (n = 10 per group): a control group, STF083010 group, PM2.5 group, and STF083010 plus PM2.5 group. The 4 groups were treated with the following reagents and dosages: 0.9% sodium chloride (NaCl) solution (B020, Nanjing jiancheng, China), 1 mg/kg STF080310 solution (HY-15845, MedChemExpress, USA), 1.5 mg/kg PM2.5 solution, and 1 mg/kg STF083010 + 1.5 mg/kg PM2.5 solution, respectively. Rats were treated with a tracheal instillation of PM2.5 or 0.9% NaCl, and at the same time received an intraperitoneal injection of STF083010 or 0.9% NaCl. The methods of administration were tracheal instillation and intraperitoneal injection, which allowed comparability among groups. All interventions in the different groups were continuously administered 5 days a week and then halted for 2 days; the total continual exposure time was 4 weeks. Experimental procedures used for all animals in this study followed the regulations and guidelines of the International Ethics Committee on Animal Welfare.

2.3. Body and organ weights

After 4 weeks of exposure, final body weights of the animals were recorded when the animals were euthanized after being anesthetized by chloral hydrate. We immediately weighed the testes and epididymides to calculate relative organ weights using the following equation: relative organ weight (%) = (wet weight of organ [g]/body weight [g]) × 100%.

2.4. Sperm quality assessment

Sperm from the cauda epididymis and vas deferens were diluted to 5 × 10^6/mL in phosphate-buffered saline and incubated at 37 °C in 5% CO_2 for further experiments. A drop of prepared sperm filtrate was evenly spread on poly-L-lysine-treated glass slides; and we then measured sperm count, sperm motility rate, and sperm malformation rate under a light microscope (E200, Nikon, Japan; 400× magnification). A computer-assisted sperm-analysis system was used to calculate the number of sperm per mL of filtrate in the epididymis, and sperm were separated based upon whether they were active or inactive. Sperm motility rate (%) = (active sperm count/total sperm count) × 100%. Sperm malformation was indexed by tail folding, double heads, double tails, broken head, no hook, and banana-shape. Sperm deformity rate

(%) = (abnormal sperm count/total sperm count) × 100%.

2.5. Determination of serum testosterone

We evaluated serum testosterone level in rats by ELISA. After the rats were sacrificed, approximately 5 mL of abdominal aortic blood was collected and subjected to centrifugation at 3000 rpm for 15 min, and the serum was carefully aspirated and stored at −20 °C for later use. According to the instructions for the rat testosterone ELISA kit (Hengyuan Biotech, Shanghai, China), we used different concentrations of testosterone standards and corresponding absorbance values to prepare a standard curve, and thus determined the testosterone in each group of samples after observing the sample absorbance.

2.6. Observation of testicular morphology

Testicular morphologic structure was observed with hematoxylin and eosin (H&E). After sacrificing the rats, single testes were taken and post-fixed in 10% formaldehyde for 48 h, followed by embedding the tissues in paraffin blocks. We then sectioned, dewaxed, dehydrated, and processed them in xylene solution. After drying the tissue sections on glass slides, the morphology of spermatogenic epithelial cells and any loss of luminal cells were observed under a light microscope at a magnification of 400×.

2.7. Detection of apoptosis in testicular tissues

An apoptotic cell detection kit (KGA703, Keygen Biotech, Nanjing, China) based on terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) was used to detect apoptotic cells in testicular tissues, according to the manufacturer's instructions. The normal cells were blue-violet in color; while the apoptotic cells exhibited a brown-chromatin concentration, apoptotic bodies, cell shrinkage, and surrounding cell separation under 400 × light microscopy. Three slices were prepared for each sample, and at least 500 cells were randomly inspected in each slice. The apoptotic index (%) = (apoptotic cell count/total cell count) × 100%.

2.8. Immunoblot analysis

The total proteins were extracted according to manufacturer's instructions (Keygen Biotech, Nanjing, China). In brief, tissue samples from testicular tissues were removed and homogenized in a lysis buffer containing 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% V/V Triton-X-100, 0.1% SDS, and protease inhibitor cocktail. Samples were then sonicated for 5 min and centrifuged at 12,000 rpm for 10 min at 4 °C. Supernatants were collected for total protein extraction, and the concentration was quantified by a bicinchoninic acid (BCA) protein assay kit (sc-278767A, Santa Cruz, USA). After separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis, we transferred the total proteins (20–30 μg) to polyvinylidene difluoride (PVDF) membranes (KGP114, Keygen Biotech, Nanjing, China). After being blocked with 5% nonfat dry milk, the membranes were incubated with the primary antibodies (1:1000) for IRE1 (27528-1-AP, Proteintech, USA), JNK (24164-1-AP, Proteintech), phosphorylated-JNK (p-JNK) (10023-1-AP , Proteintech), beclin-1 (sc-48381, Santa Cruz, USA), LC3 (sc-398822, Santa Cruz), P62 (sc-55603, Santa Cruz), and β-actin (sc-8432, Santa Cruz) at 4 °C overnight. The next day the membranes were further incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (sc-2004, Santa Cruz) at room temperature for 1 h before color development with an enhanced chemiluminescence (ECL) detection kit (KGP1123, Keygen Biotech, Nanjing, China), followed by photographing them in a Bio-Rad Gel Imaging System and analyzing the optical densities of target protein bands with the software Quantity One (Bio-Rad, Hercules, CA). β-actin was used as an internal reference to analyze the relative expression of the target proteins.

2.9. Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for comparisons among groups, with Fisher's least-significant difference (LSD) method used for pairwise comparisons between groups. GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA) was used to produce statistical graphs. A p-value <0.05 was considered significant.

3. Results

3.1. Effects of STF083010 on body and organ weights in PM2.5-treated rats

We weighed the rats after 4 weeks of exposure, and then dissected and weighed the testes and epididymides to calculate absolute and relative organ weights. As shown in Table 1, body weights and absolute organ weights in the PM2.5 group were significantly lower than in the control group ($P < 0.05$). While body and testicular/epididymal weights of rats in the STF083010+PM2.5 group were augmented, there were no significant differences compared with the PM2.5 group ($P > 0.05$). No statistical differences in relative organ weights of testes and epididymides were found among the groups ($P > 0.05$).

3.2. Effects of STF083010 on sperm quality in PM2.5-treated rats

We used an automatic sperm-quality analyzer to assess several quality parameters of rat sperm after 4 weeks of exposure. Sperm count and sperm motility rate of the rats were significantly attenuated, while sperm malformation rate was significantly higher in the PM2.5 group compared with the control group ($P < 0.01$). Sperm count and sperm motility rate were significantly higher and the sperm malformation rate was significantly lower in the STF083010+PM2.5 group compared with the PM2.5 group ($P < 0.01$) (Table 2).

3.3. Effects of STF083010 on serum testosterone in PM2.5-treated rats

Rat serum testosterone as detected by ELISA was measured in accordance with the standard curve that we prepared based on the absorbance values and concentrations of standards. The rat serum testosterone of the PM2.5 group was significantly lower than that of the control group ($P < 0.01$), while testosterone in the STF083010+PM2.5 group was significantly higher than that in the PM2.5 group ($P < 0.05$) (Fig. 1).

3.4. Effects of STF083010 on histopathologic of testicular tissues in PM2.5-treated rats

We stained representative seminiferous tubules of testes for spermatogenesis with H&E dye, followed by observations of their specific structural morphology under light microscopy (400× magnifications). As shown in Fig. 2A, the spermatogenic cells exhibited intact morphology and structure in the control group-with typically full and thick seminiferous tubules, smooth tubular walls without wrinkles, no shedding of luminal cells, and a large number of mature spermatozoa. In the STF083010 group (Fig. 2B), the seminiferous tubules showed a regular arrangement and normal structure that was composed of spermatogenic and supporting cells at different developmental stages. In the testis interstitium between the seminiferous tubules, we observed round or polygonal shapes-with large and round nuclei-in the testicular stromal cells. However, in the PM2.5 group (Fig. 2C), the structure between the spermatogenic cells was loose, with the intracellular space between the tubules appearing to be larger; the spermatogenic epithelium was degenerated; the number of mature spermatozoa decreased; and the

Table 1
Body weights and absolute and relative organ weights after 4 weeks of exposure.

Group	Body weight (g)	Testicular weight (g)	Epididymal weight (g)	Relative organ weights	
				Testis/body weight (%)	Epididymal/body weight (%)
Control	342.58 ± 19.43	1.65 ± 0.09	0.43 ± 0.04	0.48 ± 0.03	0.13 ± 0.01
STF083010	342.70 ± 11.83	1.61 ± 0.11	0.42 ± 0.03	0.47 ± 0.04	0.12 ± 0.01
PM2.5	316.27 ± 21.33*	1.50 ± 0.10*	0.37 ± 0.04*	0.47 ± 0.03	0.12 ± 0.01
STF083010+PM2.5	337.25 ± 14.45	1.62 ± 0.09	0.41 ± 0.03	0.48 ± 0.04	0.12 ± 0.01

Notes: Mean ± SD; n = 10.
* $P < 0.05$ compared with the control group.

Table 2
Analysis of sperm quality after 4 weeks of exposure.

Group	Sperm count (10^6/mL)	Sperm motility rate (%)	Sperm malformation rate (%)
Control	11.31 ± 1.54	67.07 ± 2.79	11.37 ± 1.34
STF083010	10.74 ± 1.14	67.25 ± 2.47	10.95 ± 1.53
PM2.5	6.18 ± 1.41*	35.13 ± 2.32*	26.79 ± 2.38*
STF083010 +PM2.5	8.82 ± 0.87#	50.09 ± 2.62#	22.51 ± 2.05#

Notes: Mean ± SD, n = 10.
* $P < 0.01$ compared with the control group.
# $P < 0.01$ compared with the PM2.5 group.

number of cell layers was significantly reduced compared with the control group. In contrast, in the STF083010+PM2.5 group (Fig. 2D), the structure of the spermatogenic cells was basically complete, with neatly arranged tubular cells (showing a loose but regular arrangement), and there were fewer shed cells than in the PM2.5 group. Therefore, PM2.5 exposure caused the destruction of testicular tissue morphology, and STF083010 ameliorated the PM2.5-induced pathologic damage to testicular tissues.

3.5. Effects of STF083010 on apoptosis in testicular tissues after PM2.5 exposure

Apoptosis in testicular tissues was detected by the TUNEL method and observed under a light microscopy (400× magnifications). Apoptotic cells were depicted as brown; while normal cells were stained blue-purple in color (Fig. 3A1–A4). As shown in Fig. 3B, counts of apoptotic cells and analysis of the apoptotic index for each group of testicular tissues showed that the index for the PM2.5 group was significantly higher than that of the control group ($P < 0.01$); and compared with the PM2.5 group, the apoptotic index was significantly inhibited in the STF083010+PM2.5 group ($P < 0.01$). These results suggested that PM2.5 exposure significantly increased apoptosis in testicular tissues, and that STF083010 effectively relieved PM2.5-induced apoptosis in testicular tissues.

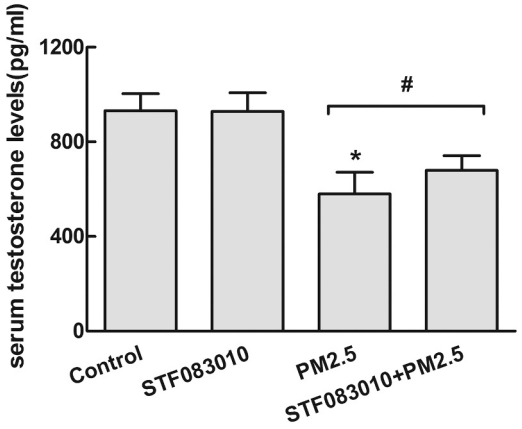

Fig. 1. Serum testosterone levels after 4 weeks of exposure. ELISA was adapted to determine the serum testosterone level (pg/mL) in the 4 groups of rats in this study. Data are presented as means ± SD, n = 10. *$P < 0.01$ compared with the control group, and #$P < 0.05$ compared with the PM2.5 group.

3.6. Effects of STF083010 on the IRE1/JNK pathway in the testis of PM2.5-treated rats

After intervention with the IRE1 inhibitor STF083010, we evaluated the expression of IRE1 cascade-related proteins (Fig. 4); and observed that IRE1 expression and JNK phosphorylation in the PM2.5 group was significantly increased ($P < 0.01$). After STF083010 intervention, the expression of IRE1 and p-JNK protein was significantly downregulated compared with the PM2.5 group ($P < 0.05$). These results showed that STF083010 significantly inhibited PM2.5-mediated testicular IRE1 expression and inhibited p-JNK upregulation while antagonizing IRE1, suggesting that the IRE1-JNK pathway may be involved mechanistically in the reproductive injury inflicted by PM2.5 exposure.

3.7. Effects of STF083010 on autophagy in the testis of PM2.5-treated rats

After intervention with the IRE1 inhibitor STF083010, we assessed the expression of autophagy-related proteins (Fig. 5). Compared with the control group, the expression of beclin-1 and LC3II/LC3I proteins of the PM2.5 group was significantly upregulated, while p62 expression was significantly downregulated ($P < 0.05$). In addition, compared with the PM2.5 group, STF083010 intervention significantly downregulated the expression of beclin-1 and LC3II/LC3I proteins ($P < 0.01$). These results indicated that PM2.5 exposure increased the level of autophagy in testicular tissues; and that STF083010 antagonized the ER stress protein IRE1 while inhibiting the expression of autophagy-related proteins. This suggested that autophagy occurred downstream of ER stress, and that PM2.5 may therefore be involved in reproductive injury in rats via the ER stress-autophagy pathway.

4. Discussion

Smog occurs frequently in China, and PM2.5 is one of the primary factors associated with the formation of smog (Zhou et al., 2015a). Thus, a key to managing smog is to resolve the problems associated with PM2.5. PM2.5 exhibits a small particle size and a large specific surface area, it allows for easy adsorption of toxicants and harmful substances such as heavy metals; and the reproductive system is one of the principal target organs manifesting toxic effects of heavy metals (Santi et al., 2016). Evidence regarding the susceptibility of the reproductive system to PM2.5 has prompted academics and scientists to conduct extensive discussions on its reproductive toxicity (Qiu et al., 2018; Yang et al., 2019). In a retrospective cohort study in Italy, investigators evaluated the effects of air pollution on sperm parameters, and demonstrated that PM2.5 exposure levels were significantly and negatively correlated with total sperm numbers (Wu et al., 2017). Analysis of the exposure-response relationship between atmospheric particulate matter and male sperm quality in Wuhan from 2013 to 2015 also showed that

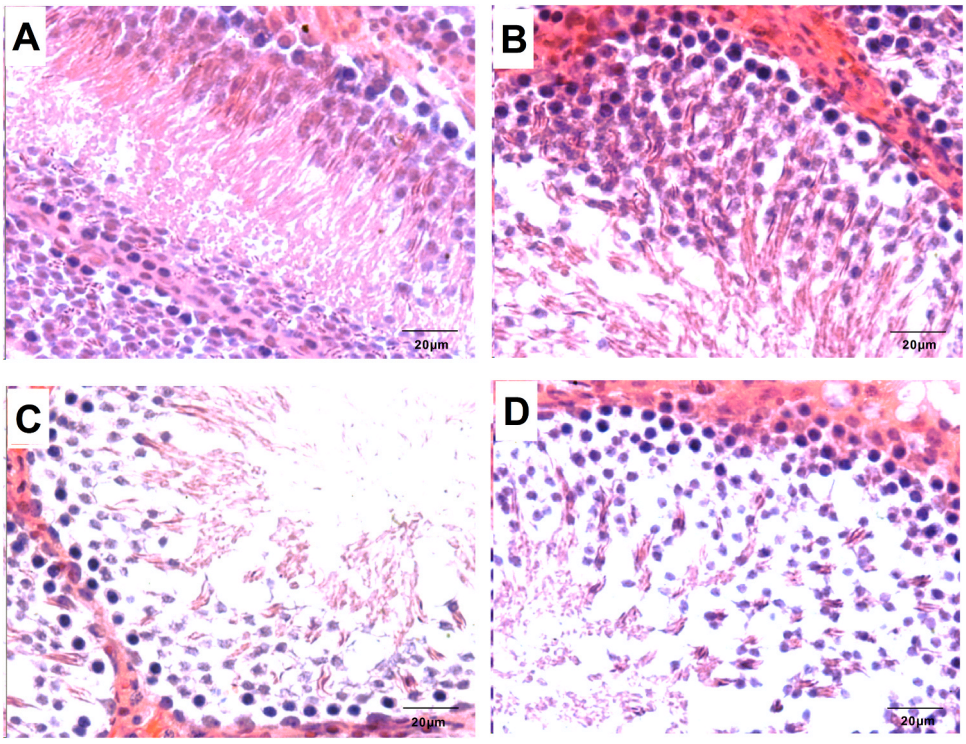

Fig. 2. Histopathologic analysis of testicular tissues after 4 weeks of exposure. The testicular seminiferous tubules of the 4 groups of rats were stained with H&E dye, and the morphologic structure of the testicular tissues in each group were observed under a light microscope (400× magnification), including the control group (A), the STF083010 group (B), the PM2.5 group (C), and the STF083010+PM2.5 group (D).

PM2.5 exposure concentration was negatively correlated with sperm quality, significantly reducing sperm concentration and sperm number (Pires et al., 2011). In the present study, 4-week-old male SD rats were exposed to PM2.5 for 4 weeks; results showed that there was a significant reduction in body and organ weights in PM2.5 group. In addition, PM2.5 reduced sperm density and sperm survival rates; increased the incidence of sperm deformities; reduced serum testosterone concentrations; damaged the structure of testicular tissues; attenuated the number of spermatogenic cells and mature spermatozoa; and augmented the apoptotic index in testicular tissues. The aforementioned lines of evidence further verified that PM2.5 caused male reproductive injury, and superimposition of STF083010 circumvented the reproductive damage caused by PM2.5 exposure. For example, after concurrent STF083010 administration, sperm quality and serum testosterone content were increased, the pathologic damage to testicular tissue was reduced, and the apoptotic index in testicular tissue was diminished.

The exact mechanisms underlying the effects of PM2.5 exposure on reproductive injury have not been fully elucidated. Some researchers showed that PM2.5 derived from automobile exhaust activated the PI3K/AKT-signaling pathway, which led to elevate levels of reactive oxygen species in testicular supporting cells and induce abnormal cellular function, obliterate the integrity of the blood–testis barrier, and ultimately lead to overall functional damage to the reproductive system (Cao et al., 2015). Establishment of a BHK-21 cell model infected with Japanese encephalitis (JE) viruses showed that inhibition of IRE1/JNK signaling was related with decreased apoptosis, indicating that the IRE1/JNK-signaling pathway plays an important role in the pathogenicity of JE viruses (Huang et al., 2016). Endoplasmic reticulum (ER) stress exhibits an important regulatory effects on spermatocytes, testicular structure, and mature sperm cells. Occurrence of severe or prolonged ER stress caused overexpression of the ER stress factor IRE1, which in turn caused activation of the JNK-signaling pathway (Kim et al., 2018). Further study is, of course, still needed to verify whether the IRE1/JNK pathway is involved in PM2.5-induced reproductive

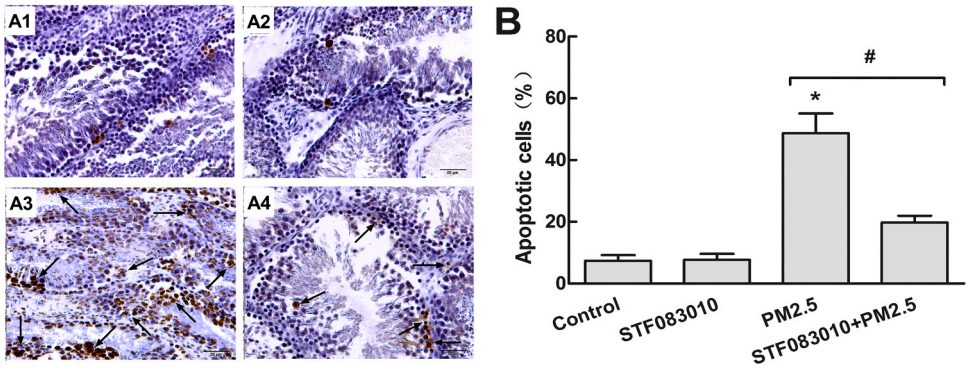

Fig. 3. Apoptotic analysis of testicular tissues after 4 weeks of exposure. Apoptosis in testicular tissues was detected using the TUNEL method and the apoptotic cells were observed under a light microscope (400 × magnification, arrows) (A1–A4); the number of apoptotic cells and apoptotic index of different groups of testicular tissues are shown in (B). Data are presented as means ± SD, n = 10. $^{*}P < 0.01$ compared with the control group, and $^{\#}P < 0.01$ compared with the PM2.5 group.

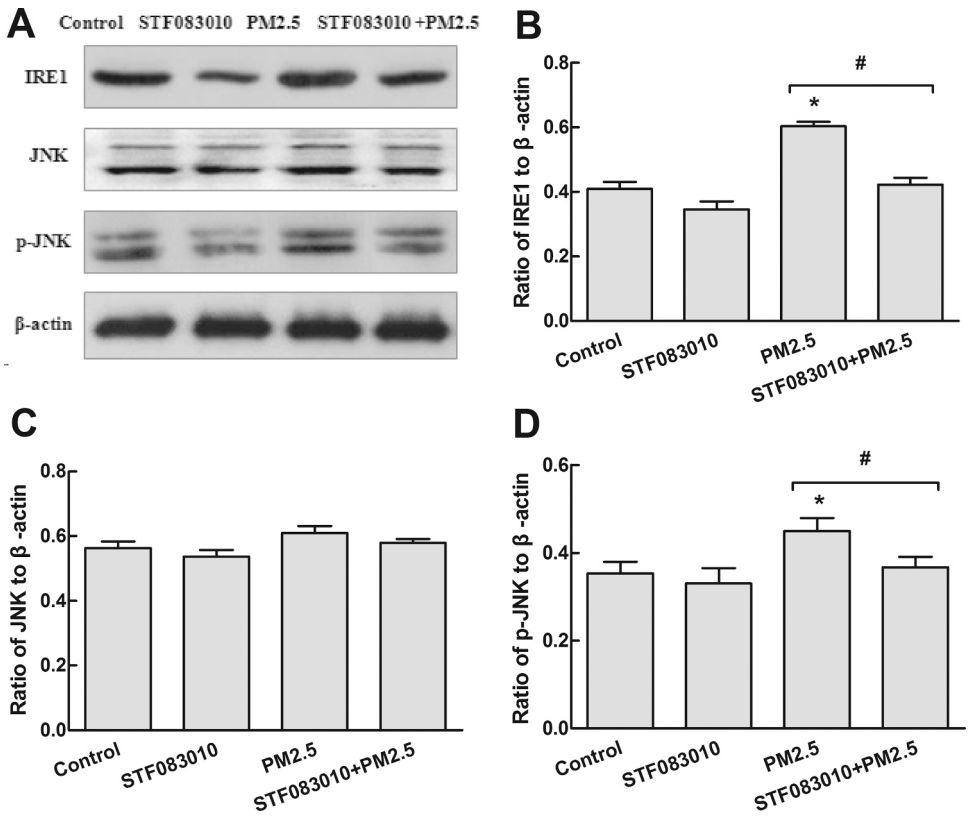

Fig. 4. Effects of STF083010 on the IRE1/JNK-signaling pathways in the testis of rats exposed to PM2.5. Western blotting was used to determine the relative expression of IRE1, JNK, and p-JNK proteins using β-actin as an internal reference (A); the ratios of target proteins to β-actin gray levels represent the relative expression of the target proteins (B–D). All values are presented as means ± SD, n = 10. $^{*}P < 0.01$ compared with the control group, and $^{\#}P < 0.05$ compared with the PM2.5 group.

injury in rats. Our study found that during PM2.5-induced reproductive injury in male rats, the expression of IRE1, JNK, and p-JNK proteins was increased; and after intervention with the IRE1 inhibitor STF083010, we observed effective suppression of JNK and p-JNK protein expression-suggesting that IRE1 may participate in PM2.5-induced reproductive injury in male rats by activating JNK signaling.

Autophagy may promote or inhibit diseases, and abnormal autophagic function plays an important role in the occurrence and progression of disease (Xu et al., 2019). There are only a few published studies on the relationship between autophagy and PM2.5-induced reproductive damage, and it is therefore important to explore the role of autophagy in PM2.5-induced male reproductive injury (Wang and Tang, 2020). In a previous study investigators used PM2.5 to stimulate human nasal epithelial cells, and transmission electron microscopy showed that the autophagosome and autolysosome were increased significantly, and that the expressions of LC3II and beclin-1 proteins were also increased (Zhao et al., 2019). We know that the process of autophagy is strictly regulated from a molecular standpoint. In addition to autophagy mediated by the two classical pathways, i.e. the mTOR and PI3K/Akt signal transduction pathways, ER stress-induced autophagy has recently developed into a promising area of research (Utaipan et al., 2017). Beclin-1 is the first found key protein found to initiate autophagy in mammals, and is also known to be a basic overall regulator of autophagy (Oberstein et al., 2007). In the present study, PM2.5 exposure caused a significant upregulation of beclin-1 and LC3 protein expression and a significant downregulation of p62 protein in rat testicular tissues. The use of STF083010, then, inhibited IRE1 and reduced the expression of the autophagy-related proteins, suggesting that autophagy occurred downstream of the ER stress factor IRE1-which may then play an important role in PM2.5-induced reproductive injury in male rats by regulating autophagy.

5. Conclusion

In summary, the present study has shed some light on the toxic mechanism of PM2.5. Our results showed that the ER stress-autophagy signaling is a crucial mechanistic event involved in reproductive toxicity, and the IRE-JNK pathway is also a major regulator of autophagy activation. These findings advance our understanding of PM2.5 toxicity and provide potential molecular targets for preventing reproductive injury from PM2.5 exposure. Mitochondrial dysfunction may play an important role in reproductive toxicity. Key mitochondrial functions were perturbed by particulate matter exposure in human olfactory mucosal cells (Chew et al., 2020). The autophagy and ER stress are all mediated by mitochondrial dysfunction in the pathogenesis of acute pancreatitis (Biczo et al., 2017). Future studies may focus on the crosstalk between mitochondrial signaling and ER stress-autophagy signaling. In addition, JNK is extensively involved in physiologic and pathologic reactions within cells. Studies have shown that activated JNK induces autophagy by causing phosphorylation of p53 or Bcl-2 (Lu et al., 2017). However, little is known regarding the detailed of how JNK triggers autophagy in PM2.5-induced reproductive injury. This is another important topic for future research.

CRediT authorship contribution statement

Yang Yang: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Writing - review & editing. **Yajing Feng:** Data curation, Formal analysis, Investigation, Resources. **Hui Huang:** Project administration, Resources, Writing - review & editing. **Liuxin Cui:** Conceptualization, Formal analysis. **Fuqin Li:** Conceptualization, Project administration. All authors have read and agreed to the published version of the manuscript.

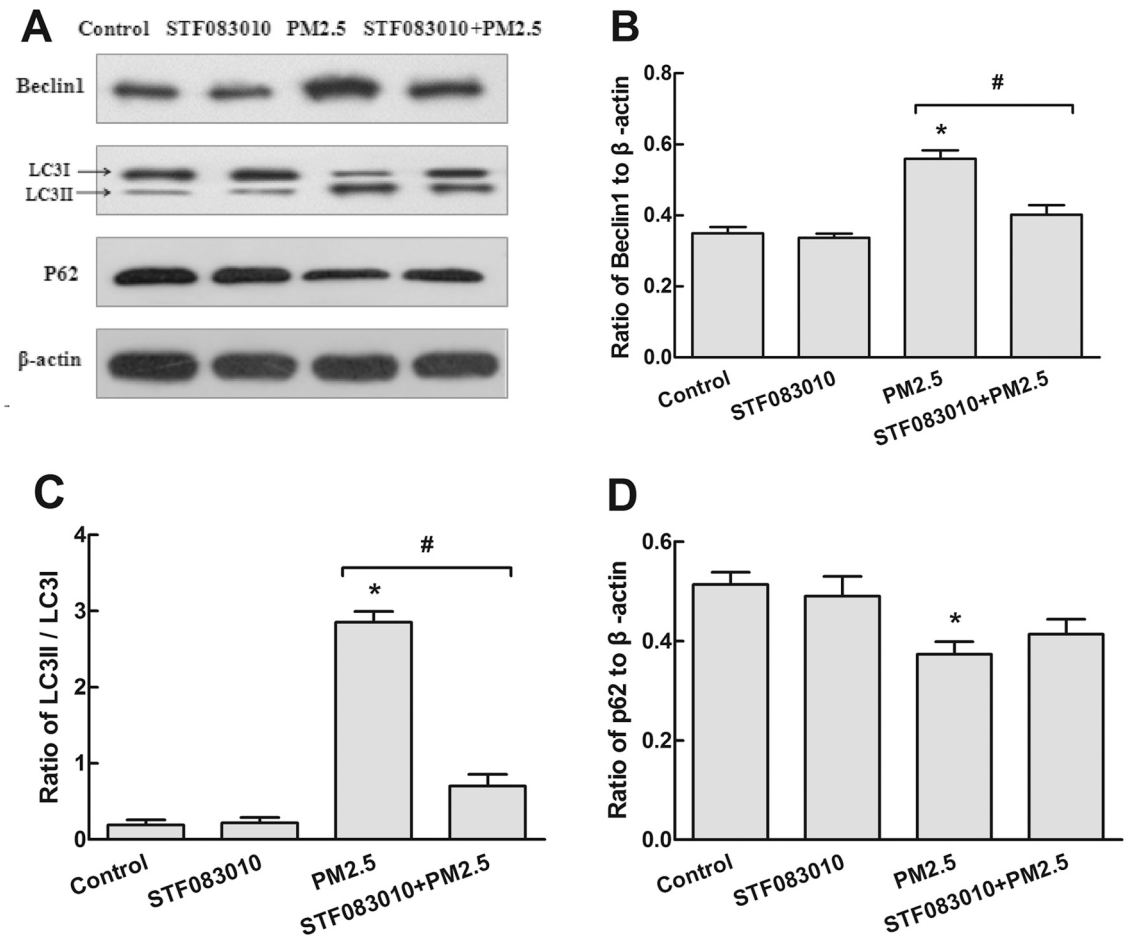

Fig. 5. Effects of STF083010 on the level of autophagy in the testes of rats exposed to PM2.5. Western blotting was used to determine the relative expression of autophagy-related proteins in rat testis-including beclin-1, LC3, and p62-using β-actin as an internal reference (A); the ratios of target proteins to β-actin gray levels represent the relative expression of the target proteins (B–D). All values are presented as means ± SD, n = 10. $^{*}P < 0.05$ compared with the control group, and $^{\#}P < 0.01$ compared with the PM2.5 group.

Declaration of Competing Interest

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

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