Efficacy and mechanism of lipoic acid in the treatment of reproductive injury caused by perfluorooctanoic acid

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Abstract. Environmental pollutants, including perfluorooctanoic acid (PFOA), cause severe reproductive damage to humans and animals. Lipoic acid (LA) is a strong antioxidant that alleviates the oxidative damage caused by heavy metals, environmental toxicants, chemical poisons, etc. Therefore, the present study investigated the curative effect and mechanism of LA in treating spermatogenesis dysfunction caused by PFOA. A rat reproductive injury model was established by gavage of PFOA for consecutive 30 days and then rats were treated with different doses of LA for 42 days. The effects were assessed by ELISA, western blotting, H&E staining and immunofluorescence staining. The results demonstrated that LA had ameliorating effects on PFOA-induced reproductive injury in rats, it increased the sperm counts, and the levels of serum succinate dehydrogenase, lactate dehydrogenase, glutathione peroxidase and superoxide dismutase returned to normal levels following LA treatment. In addition, LA promoted the expression of estradiol, reduced the expression of serum sex hormones, such as follicle-stimulating hormone, androgen receptor (AR) and malondialdehyde in the testes, and restored the structure of seminiferous tubules. Its therapeutic mechanisms include regulating the testicular oxidative stress pathway and hypothalamic-pituitary-testis axis.

Introduction

Perfluorooctanoic acid (PFOA) is a newly discovered organic environmental pollutant distributed in nature, and it has been widely used in the fields of surfactants, emulsifiers, textiles and interior decoration in the past few decades (1). Due to the characteristics of refractory degradation and bioaccumulation, it has been detected in animals, plants and water, causing serious pollution to the environment (2,3). Previous studies have demonstrated that PFOA is harmful to the nervous, immunity and endocrine systems, and particularly the reproductive system (4-7). PFOA binds to oestrogen receptors and causes oxidative damage to the body, thereby impairing male reproductive function (8).

Lipoic acid (LA) with the chemical name 1,2-dithiolane-3-valeric acid is a strong antioxidant, widely present in living organisms and serves a role in scavenging oxygen free radicals and chelating metal ions (9). It alleviates the oxidative damage caused by heavy metals, environmental toxicants or other factors (10). It also possesses therapeutic effects in the treatment of diabetes, peripheral neuropathy, male infertility disease, etc. (11-13). The present study further explored whether LA could improve the spermatogenesis disorder induced by PFOA in rats. Male Sprague Dawley (SD) rats were given 0.01 g/kg PFOA by gavage for 30 consecutive days to establish a PFOA-induced rat model. Subsequently, rats with reproductive damage were treated with either a high or low dose of LA. The therapeutic efficiency and mechanism of action were examined by western blotting, H&E staining, ELISA and immunofluorescence techniques.

Materials and methods

Materials. A total of 30 specific pathogen-free 6-week-old male SD rats were purchased from SPF (Beijing) Biotechnology Co., Ltd. and their weights were ranged between 150 and 170 g. PFOA (96%; white powder) was purchased from Shanghai Anpu Experimental Technology Co., Ltd. LA was purchased from Sigma-Aldrich; Merck KGaA. Anti-rabbit β-actin (cat. no. sc-47778) was purchased from Santa Cruz Biotechnology, Inc. SDS-PAGE gel, BCA, RIPA lysis buffer, antifade mounting medium with DAPI (cat. no. P0131), immuno staining blocking buffer (cat. no. P0102) and H&E staining kits were all purchased from Beyotime Institute of Biotechnology. EZ-Link Sulfo-NHS-LC-Biotin (cat. no. 21335) and streptavidin-fluorescein conjugate (cat. no. S869) were purchased from Invitrogen; Thermo Fisher Scientific, Inc. The rat follicle-stimulating hormone (FSH; cat. no. m1059034), rat testosterone (T; cat. no. ml059506), rat lactate dehydrogenase

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(LDH; cat. no. m1059178), rat malondialdehyde (MDA; cat. no. m1077384) and rat serum succinate dehydrogenase (SDH; cat. no. ml058919), rat superoxide dismutase (SOD; cat. no. m1059387), rat glutathione peroxidase (GSH-Px; cat. no. m1097316), rat luteinizing hormone (LH; cat. no. m1002860) and rat estradiol (E2; cat. no. m1002871) kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. Neutral balsam (cat. no. G8590) was purchased from Beijing Solarbio Science & Technology Co., Ltd.

Animal grouping and treatments. After 1 week of adaptive feeding, the rats were randomly divided into five groups according to their body weight based on a random number table, with 6 rats in each group. These groups were: Control group, PFOA-induced group (PFOA), LA control group (LA), PFOA-induced low-dose LA medication group [PFOA+LA (L)] and PFOA-induced high-dose LA medication group [PFOA+LA (H)]. LA and PFOA were dissolved in corn oil (0.2% of rat body weight), and all the treatments were administered by gavage at 10 a.m. every day. The duration of LA treatment was 6 weeks. The control group was given corn oil continuously for 72 days, the PFOA-induced group was given 0.01 g/kg PFOA for 30 days and then euthanized after detection was carried out, the LA control group was given 0.01 g/kg LA for 42 days and then euthanized after detection was carried out, the PFOA+LA (L) group was given 0.01 mg/kg PFOA for 30 days to establish the model and then given the treatment of 0.05 g/kg LA for 42 days, and the PFOA+LA (H) group was given 0.01 mg/kg PFOA for 30 days to establish the model and then given the treatment of 0.10 g/kg LA for 42 days. Rats had free access to water and food. The room temperature and relative humidity of the animal laboratory were 20-25˚C and 40-60%, respectively, with a 12/12 h day-night cycle. The body weights of rats were measured and recorded every weekend, and the weight of rats at the time of euthanasia was ~350 g. After 30 days of gavage of PFOA solution and 42 days of LA treatment, blood was collected from the abdominal aorta of rats anesthetized by inhalation of 4% ether and the supernatant was reserved after centrifugation at 4˚C for 5 min at 600 x g. Rats were not allowed to live on after blood collection or biotin-labelled blood-testis barrier (BTB) function analysis. Euthanasia by cervical dislocation was performed immediately after the experiment was completed. In addition, the other rats in each group were sacrificed by cervical dislocation after anaesthesia by inhalation of 4% ether, their testes and epididymis were quickly extracted and weighed with a balance. Tissues were homogenized or sliced after fixation in 4% paraformaldehyde at room temperature (25˚C) for 15 min. Absence of movement and breath, as well as the presence of cardiac arrest and pupil dilation for 5 min were used to confirm death. Animal experiments and treatments were performed in accordance with the guidelines of the experimental animal management and ethics committee of Baotou Medical College (Baotou, China) and humane care was given according to the 3R principle.

BTB function test. A total of 2 rats from each group were anesthetized by inhalation of 4% ether and their testis tissues were exposed after disinfection with alcohol. A small opening was created with surgical scissors to inject a biotin solution (50 μl EZ-Link Sulfo-NHS-LC-Biotin 10 mg·ml^{-1} in 1X PBS; cat. no. 21335; Invitrogen; Thermo Fisher Scientific, Inc.) into the testes of the rat and it was left to disperse for 30 min at room temperature. Subsequently, the rat testis tissues were rapidly isolated and placed in liquid nitrogen (-196˚C) for preservation, and rats were sacrificed by cervical dislocation immediately. Testis sections (thickness, 8 μm) were obtained from different levels at -20˚C. Then sections were incubated with streptavidin-fluorescein conjugate (1:3,000; cat. no. S869; conjugate, fluorescein; Invitrogen; Thermo Fisher Scientific, Inc.) for 30 min in the dark after blocking with immuno staining blocking buffer for 2 h (all at room temperature). The sections were rinsed with PBS and mounted in a drop of antifade mounting medium with DAPI at room temperature. An Olympus BX51 fluorescence microscope (Olympus Corporation) was used for imaging. The Cell sens Dimension software (V4.1; Olympus Corporation) was used for analysis.

Testicular H&E staining. The testis tissues of rats were quickly extracted and fixed in 4% paraformaldehyde solution at room temperature for 24 h. Subsequently, the testis tissues were embedded in paraffin and cut into tissue sections (thickness, 5 μm). The sections were incubated in xylene for 10 min, absolute ethanol for 5 min, 95% alcohol for 30 min, 90% alcohol for 30 min, 80% alcohol for 30 min, 70% for alcohol 30 min, and then rinsed in distilled water for 5 min at room temperature for dewaxing. Sections were stained with hematoxylin for 4 min, rinsed with water for 10 min, differentiated with hydrochloric acid ethanol for 3 sec, stained with eosin for 1 min and then rinsed with deionized water for 5 min, and finally sealed with neutral balsam after air-drying naturally (all at room temperature). An Olympus BX51 fluorescence microscope (Olympus Corporation) was used for imaging. The Cell sens Dimension software (V4.1; Olympus Corporation) was used for analysis.

Sperm extraction and counting. The rat bilateral epididymis was stripped into an Eppendorf tube containing 1.5 ml pre-warmed PBS (37˚C) and incubated for 20 min on a shaker to completely dissociate the sperm into the PBS buffer. Subsequently, the semen was filtered with a 70-μm membrane filter and rinsed with 1 ml PBS simultaneously. A total of 300 μl 5% $NaHCO_3$ was mixed with the aforementioned semen. The cells in the suspension were counted using a hemacytometer and Olympus IX51 fluorescence microscope (Olympus Corporation) after the cell suspension was left to stand for 1 min to allow the cells to settle on the hemacytometer. Each sample was counted three times and the mean value was taken.

Detection of SDH, LDH, SOD, MDA and GSH-Px activity, and T, FSH, LH and E2 levels in testes. The rat testis was dried with filter paper, cut into small pieces on ice, and then ground into 10% homogenate mechanically, and the supernatant was collected after centrifugation at 4˚C for 5 min at 500 x g. The detection operation was performed using the corresponding ELISA kits according to the manufacturer's instructions and the absorbance value was detected using a microplate reader.

Western blotting. Total protein from rat testis tissues was extracted using RIPA lysis buffer and quantified using a BCA assay. Total protein (20 μg/lane) was separated by 10% SDS-PAGE and

Table I. Therapeutic effects of LA on organ coefficients of rats.

Group	Dose, g/kg	Weight, g	Weight of testis, g	Weight of epididymis, g	Weight coefficient of testis, ‰	Weight coefficient of epididymis, ‰
Control	-	474.87±38.48	3.63±0.10	0.84±0.05	7.30±0.57	1.70±0.10
PFOA	0.01	411.84±33.23[a]	3.66±0.20	0.77±0.02[a]	9.30±0.45[a]	1.90±0.18[a]
LA	0.10	469.17±32.24[b]	3.68±0.24	0.89±0.08[b]	8.00±0.63[b]	1.80±0.18
PFOA+LA (L)	LA, 0.05; PFOA, 0.01	405.99±23.05[a,c]	3.72±0.14	0.77±0.04[a,c]	9.00±0.85[a,c]	1.80±0.11
PFOA+LA (H)	LA, 0.10; PFOA, 0.01	428.24±23.68[a-d]	3.55±0.23	0.83±0.04[b-d]	8.90±0.66[a-c]	1.90±0.90[a]

[a]P<0.05 vs. control; [b]P<0.05 vs. PFOA; [c]P<0.05 vs. LA; and [d]P<0.05 vs. PFOA+LA (L). Data are presented as the mean ± standard deviation (n=6). LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

transferred onto a PVDF membrane that was washed twice with PBS with 0.05% Tween-20 (PBST) and subsequently blocked at 25˚C for 2 h with 5% skimmed milk after two washes with PBST. The membrane was subsequently incubated with primary antibodies against androgen receptor (AR; 1:2,000; ab133273; Abcam) and β-actin (1:2,000) overnight at 4˚C. Following incubation with primary antibodies, membranes were washed three times with PBST and incubated with goat anti-rabbit IgG (H+L) secondary antibody (conjugate, DyLight® 488; 1:3,000) for 2 h at 25˚C. The protein bands were visualized after exposure to enhanced chemiluminescence solution (SuperSignal™ West Atto; A38555; Thermo Fisher Scientific, Inc.) on a Tanon-4600 image analysis system (Tanon Science and Technology Co., Ltd.). The gray value of each band was calculated and analyzed using ImageJ software (National Institutes of Health).

Statistical analysis. All assays were repeated at least three times, and values are presented as the mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance followed by Tukey's test as post hoc tests using SPSS 17.0 statistical software (SPSS, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

LA has an ameliorating effect on physical damage caused by PFOA. Compared with those of the rats in the control and LA groups, the body and epididymal weights of the rats in the PFOA-induced group were substantially decreased. The body and epididymal weights of the rats in PFOA+LA (H) groups were increased, but there was no change in the rats in the PFOA+LA (L) group. In addition to body weight, high doses of LA also served a role in restoring the sex organ coefficients such as the weight coefficient of testis compared with the PFOA-induced group (Table I). The treatment with high dose of LA increased the epididymal mass of PFOA-induced rats to the normal weights of the rats in the control group.

LA has a therapeutic effect on BTB in PFOA-induced rats. BTB is an immune barrier composed of Sertoli cells and surrounding structures, which provides a reliable guarantee for spermatogenesis (14). After being induced by PFOA, the structure of the seminiferous tubule was destroyed. Biotin diffused farther, the number of spermatogenic cells was decreased and the gap between spermatogenic cells was increased. After treatment with LA, the structural damage of seminiferous tubules was alleviated. Furthermore, the diffusion distance of biotin in the seminiferous tubule lumen was reduced, indicating that the physiological state of the BTB had been restored (Fig. 1).

LA has a therapeutic effect on testis tissues of PFOA-induced rats. H&E staining demonstrated that the seminiferous tubules in the testis tissue of normal rats were intact and closely arranged. The spermatogenic cells in the seminiferous tubules were morphologically regular and distinct, and the formation of sperm was visible. In contrast to the control group, the seminiferous tubule lumen in the PFOA-induced rats was narrowed, and the seminiferous epithelium was thinned. The arrangement of spermatogenic cells was disordered, and numerous immature spermatogenic cells fell off into the seminiferous tubule lumen. At the same time, Sertoli cells and stromal cells were vacuolated. The number of spermatogenic cells was increased after treatment with low-dose LA. The histopathology of testes was restored after treatment with high-dose LA. In addition, the shapes of seminiferous tubules became more regular, and the spermatogenic cells were closely arranged with distinct layers (Fig. 2). Staining results indicated that LA alleviates the PFOA-induced damage to the testes in rats.

LA relieves spermatogenesis disorder caused by PFOA. Sperm count is an important indicator of reproductive toxicology, which accurately reflects the degree of reproductive damage to animals caused by toxicants (15,16). The sperm counts of rats were reduced after PFOA intoxication compared with the counts of rats in control and LA groups. After treatment with LA, the symptoms were relieved to varying degrees, and the effect was positively related to the dose (Table II).

LA activates the expression of testicular marker enzymes. The levels of SDH and LDH in the serum of rats were decreased after PFOA intoxication. This indicated that PFOA damaged

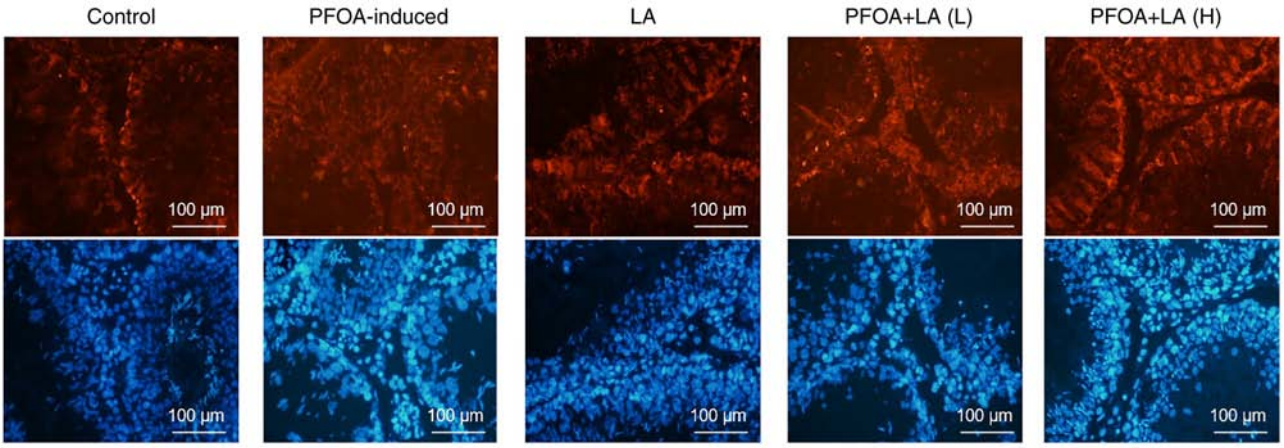

Figure 1. LA has protective effects on the BTB in PFOA-induced rats. Representative outcomes of the BTB function test showing that the structural damage of seminiferous tubules was alleviated and the diffusion distance of biotin in the seminiferous tubule lumen was reduced after treatment with LA. The top row shows the diffusion distance of biotin in the seminiferous tubule lumen under different treatments. The bottom row shows DAPI staining of cells. Scale bar, 100 μm. BTB, blood-testis barrier; LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

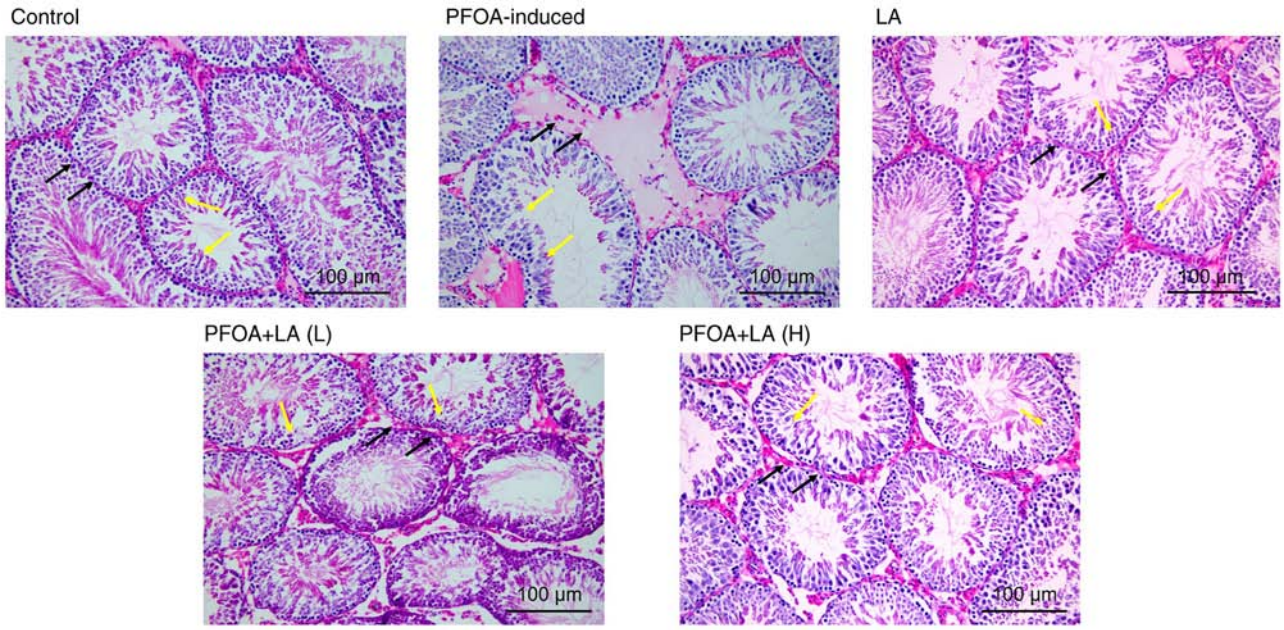

Figure 2. LA alleviates PFOA-induced damage to testicular tissue in rats. Representative images of testicular H&E staining showing that seminiferous tubules returned to normal shape, and the spermatogenic cells became tightly connected after treatment with LA. Scale bar, 100 μm. Black arrow=seminiferous tubules; yellow arrow=spermatogenic cells. LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group; H&E, hematoxylin and eosin.

the production of energy and sperm in the testis tissues. After treatment with high-dose LA, the levels of SDH and LDH in the serum of rats in PFOA+LA (H) were increased compared with those in the PFOA-induced group. The therapeutic effect was positively associated with the dose of LA administered. Therefore, LA alleviated serum hormone disorder in rats caused by PFOA (Table III).

LA restores the expression of hormones in PFOA-induced rats. The present study elucidated the mechanism of LA in treating reproductive injury caused by PFOA by detecting the changes in FSH, T, E2 and LH levels in rat serum (Table IV). The levels of FSH in rats were significantly increased after PFOA induction, while the levels of T and E2 were decreased ($P<0.05$). After treatment with LA, the levels of FSH were decreased and those of E2 were increased to varying degrees compared with the PFOA-induced group, and the therapeutic effect was positively associated with the dose of LA. In addition, T levels of rats in the PFOA+LA (H) group returned to the levels of control group. These results demonstrated that LA possessed the ability to restore the expression of sex hormones in PFOA-induced rats.

LA attenuates the oxidative stress injury induced by PFOA in rats. The activities of SOD and GSH-Px in the testis tissues were decreased after PFOA induction, while the

Table II. Therapeutic effects of LA on sperm counts in PFOA-induced rats.

Group	Dose, g/kg	Counts, x10^6
Control	-	22.50±3.21
PFOA	0.01	11.50±1.52[a]
LA	0.10	19.00±5.10[b]
PFOA+LA (L)	LA, 0.05; PFOA, 0.01	15.82±1.72[a,b]
PFOA+LA (H)	LA, 0.10; PFOA, 0.01	17.50±1.26[b]

[a]P<0.05 vs. control; [b]P<0.05 vs. PFOA. Data are presented as the mean ± standard deviation (n=6). LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

Table III. Therapeutic effects of LA on testicular marker enzymes.

Group	Dose, g/kg	Serum succinate dehydrogenase, U/mg	Lactate dehydrogenase, U/g
Control	-	1.56±0.31	2.70±0.59
PFOA	0.01	1.00±0.41[a]	2.10±0.49[a]
LA	0.10	1.73±0.43[b]	2.61±0.89[b]
PFOA+LA (L)	LA, 0.05; PFOA, 0.01	1.54±0.40[b,c]	2.12±0.54[a]
PFOA+LA (H)	LA, 0.10; PFOA, 0.01	1.71±0.31[a,b,d]	2.42±0.53[b]

[a]P<0.05 vs. control; [b]P<0.05 vs. PFOA; [c]P<0.05 vs. LA; and [d]P<0.05 vs. PFOA+LA (L). Data are presented as the mean ± standard deviation (n=6). LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

production of MDA was increased. Treatment with high-dose LA increased the expression of SOD and GSH-Px and decreased the production of MDA, but there was no difference in the rats in the PFOA+LA (L) group. The therapeutic effect was dose-related. These results demonstrated that LA alleviated the oxidative stress injury caused by PFOA (Table V).

LA reduces AR expression in PFOA-induced rats. Western blotting results revealed that AR protein expression in rat testes was increased after PFOA induction (Fig. 3). The increase in AR expression has been reported be associated with the destruction of the hypothalamus-pituitary-testis axis (17). AR expression was decreased after administration of LA; however, no significant difference was observed in the rats in the PFOA+LA (L) group, and the therapeutic effect was positively associated with the dose of LA.

Discussion

The extensive use of PFOA has caused serious pollution to the environment (3). PFOA reduces the levels of human serum T and reproductive hormones, resulting in delayed embryonic development or miscarriage during pregnancy (18-21). LA is an important cofactor in mitochondrial metabolism, and it can scavenge a variety of oxygen free radicals (22,23). Therefore, the present study aimed to explore whether LA has a therapeutic effect on PFOA-induced reproductive damage.

The integrity of the BTB is an important condition to ensure the progress of spermatogenesis (14). In addition, the body weights and sperm counts are indicators of animal reproductive toxicity (24). After being induced by PFOA, the rats exhibited destroyed seminiferous tubule structure, decreased sperm counts, decreased weights of testes and epididymis, irregular shape of seminiferous tubules, disordered arrangement of spermatogenic cells, and decreased numbers of spermatogenic cells. These symptoms were alleviated after the intervention of LA, and the therapeutic effect was positively associated with the dose.

LDH and SDH serve an important role in the process of spermatogenesis and can be used as indicators to evaluate spermatogenesis (25,26). SDH exists in seminiferous tubules and mitochondria of spermatogenic cells, and it can convert sorbitol to fructose (27). Fructose generates energy through the glycolysis pathway in sperm and participates in the energy metabolism of sperm (28). LDH is the key enzyme of sperm glycolysis, and it participates in the development of spermatogenic cells and the energy metabolism of sperm (27,29). After treatment with high-dose LA, the activities of SDH and LDH returned to normal levels, which alleviated the testicular energy metabolism disorder caused by PFOA in rats.

When the testis is stimulated by external stimuli or harmful substances, it will generate a defensive response, and the testis will produce excess reactive oxygen species (ROS) (30). The excessive ROS produced exceed the antioxidant scavenging capacity of the testis, causing peroxidative damage to the testis (31). MDA is a product of lipid peroxidation and its amount can be used as a marker to assess oxidative damage (32). In addition, SOD and GSH-Px are indispensable antioxidant defence enzymes in the testes (33). The present study revealed that the production of MDA in the testis tissues of PFOA-induced rats was increased, while the activities of SOD and GSH-Px were decreased. After LA treatment, the production of MDA was decreased, and the activities of SOD and GSH-Px were increased, indicating that LA had a therapeutic effect on PFOA-induced testicular tissue peroxidation.

Furthermore, the present study explored the mechanism of LA in treating reproductive injury induced by PFOA at the hormone level. The hypothalamus-pituitary-testis axis is closely related to spermatogenesis (34). The hypothalamus secretes a gonadotropin-releasing hormone that acts on the pituitary, prompting the pituitary to secrete FSH (35,36). FSH not only binds to receptors on Sertoli cells to regulate spermatogenesis and differentiation of spermatogenic cells but also promotes Leydig cells to synthesize T (17). AR mainly exists in the Sertoli cells of the testes and is a necessary intermediate substance for androgen to serve the role of spermatogenic regulation (18,37). T serves a role in male reproduction by

Table IV. Therapeutic effects of LA on hormone levels.

Group	Dose, g/kg	Follicle-stimulating hormone, pg/ml	Testosterone, pg/ml	Estradiol, pg/ml	Luteinizing hormone, U/ml
Control	-	0.75±0.02	2.82±0.04	292.22±71.85	10.50±1.03
PFOA	0.01	0.89±0.01[a]	2.72±0.03[a]	156.15±37.40[a]	8.91±1.85[a]
LA	0.10	0.74±0.04[b]	2.80±0.03[b]	290.00±70.56[b]	9.89±1.01
PFOA+LA (L)	LA, 0.05; PFOA, 0.01	0.81±0.03[a,c]	2.74±0.03[a,c]	183.99±25.02[a,c]	9.30±1.58[a]
PFOA+LA (H)	LA, 0.10; PFOA, 0.01	0.77±0.02[b,d]	2.77±0.02[b]	246.27±38.92[a-d]	9.82±1.90[b]

[a]P<0.05 vs. control; [b]P<0.05 vs. PFOA; [c]P<0.05 vs. LA; and [d]P<0.05 vs. PFOA+LA (L). Data are presented as the mean ± standard deviation (n=6). LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

Table V. Therapeutic effects of LA on oxidative injury.

Group	Dose, g/kg	Superoxide dismutase, U/ml	Glutathione peroxidase, U/mg	Malondialdehyde, nmol/mg
Control	-	18.86±1.37	722.88±118.17	0.87±0.14
PFOA	0.01	9.60±0.80[a]	306.20±77.53[a]	1.24±0.35[a]
LA	0.10	18.82±1.34[b]	720.78±116.52[b]	0.85±0.16[b]
PFOA+LA (L)	LA, 0.05; PFOA, 0.01	11.24±1.63[a,c]	396.77±101.24[a,c]	0.98±0.15
PFOA+LA (H)	LA, 0.10; PFOA, 0.01	17.18±1.42[b,d]	544.58±89.41[a-d]	0.70±0.23[b]

[a]P<0.05 vs. control; [b]P<0.05 vs. PFOA; [c]P<0.05 vs. LA; and [d]P<0.05 vs. PFOA+LA (L). Data are presented as the mean ± standard deviation (n=6). LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

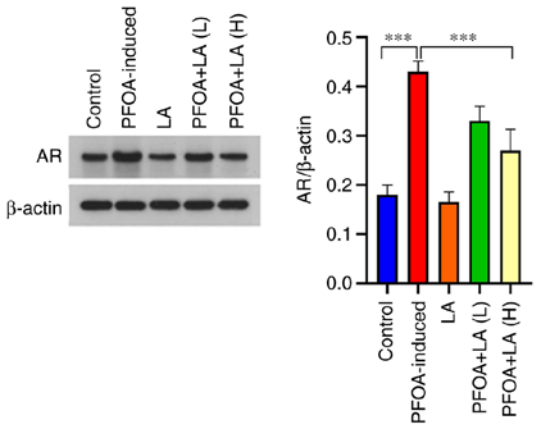

Figure 3. LA treatments reduces AR expression. (Left) Representative western blot images showing the levels of AR protein in rat testes measured in different experimental groups. (Right) Western blot analysis AR protein semi-quantification for different experimental groups. ***P<0.01. AR, androgen receptor; LA, lipoic acid; PFOA, perfluorooctanoic acid; PFOA+LA (H), PFOA-induced high-dose LA medication group; PFOA+LA (L), PFOA-induced low-dose LA medication group.

affecting the synthesis of E2 (38). The levels of FSH were increased, and the levels of T and E2 were decreased in the PFOA-induced rats. However, the levels of LH in the plasma did not change after induction of PFOA, which is consistent with the results of another study (39). This result may be caused by PFOA disrupting the rat hypothalamic-pituitary-testis axis to initiate the feedback regulation of FSH. Western blotting results demonstrated that AR expression was increased after exposure to PFOA. The levels of serum hormone and AR expression in rats returned to normal after LA treatment, indicating that LA exerted therapeutic effects via the hypothalamic-pituitary-testis axis.

In summary, LA was effective in treating PFOA-induced reproductive damage by regulating the oxidative stress pathway in the testes and the hypothalamic-pituitary-testis axis. LA may be applied to humans to protect the reproductive system against poisons in the future.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JB, ZZ and RW participated in the conception, design and data acquisition of the article. WZ and NG participated in the analysis and interpretation of data, drafting the manuscript and made critical revisions for important intellectual content. JB ensured that questions related to the integrity of any part of the work were appropriately investigated and resolved. ZZ, RW, WZ, NG and JB confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Baotou Medical College, Inner Mongolia University of Science and Technology (Baotou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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