Research Article

Baicalein Alleviates Testicular Ischemia-Reperfusion Injury in a Rat Model of Testicular Torsion-Detorsion

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Testicular torsion/detorsion-induced ischemia/reperfusion injury is partly due to the overgeneration of reactive oxygen species. Baicalein, a main bioactive constituent derived from the dried root of Scutellaria baicalensis Georgi, possesses powerful antioxidative and anti-inflammatory properties. Therefore, we designed the research to explore the possible protective effect of baicalein against testicular ischemia-reperfusion injury. Sprague-Dawley rats were randomized into 4 groups, including control, testicular ischemia-reperfusion, testicular ischemia-reperfusion+vehicle injection, and testicular ischemia-reperfusion+baicalein therapy groups. The control group received surgical exposure of the left testis without torsion-detorsion. In the testicular ischemia-reperfusion group, the left testis underwent 720° counterclockwise torsion for two hours and then was allowed detorsion. Rats in the testicular ischemia-reperfusion+vehicle injection group received intraperitoneal injection of the vehicle at detorsion. In the baicalein-treated group, the intraperitoneal administration of baicalein dissolved in the vehicle was performed at detorsion. At four hours or three months following testicular detorsion, testicular tissues were removed to detect the levels of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) which can recruit neutrophils into the testis, myeloperoxidase activity (an index of neutrophil infiltration in the testis), protein expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in neutrophils which can catalyze reactive oxygen species production, malondialdehyde concentration (a common marker of reactive oxygen species), and spermatogenesis. Both testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle injection significantly increased the TNF-α and IL-1β levels, myeloperoxidase activity, NADPH oxidase protein expression, and malondialdehyde concentration, while decreased spermatogenesis in ipsilateral testes. In contrast, baicalein administration remarkably reduced TNF-α and IL-1β secretion, neutrophil infiltration in the testis, and NADPH oxidase protein expression in neutrophils to reduce reactive oxygen species production.

1. Introduction

Testicular torsion caused by rotation of the spermatic cord around itself impedes testicular blood flow. Prolonged ischemic duration of the testis can result in testicular necrosis. The most common treatment method for testicular torsion is early surgical detorsion. It recovers testicular blood circulation and prevents ischemic necrosis. However, despite timely detorsion, testicular atrophy still occurs in 9.1%-73.3% of patients in the long-term follow-up [1, 2]. Damage caused by testicular torsion-detorsion is an ischemia-reperfusion injury. In the process of ischemia-reperfusion, excessive reactive oxygen species are formed in tissue [3–5]. Reactive oxygen species, like superoxide anions, nitric oxide, hydrogen peroxide, hypochlorous acid, hydroxyl radicals, and singlet oxygen, are highly reactive and can damage cellular constituents, such as lipids, proteins, deoxyribonucleic acid, and

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carbohydrates, finally leading to the loss of cellular viability and even cellular death [6]. Testicular tissue is very sensitive to reactive oxygen species because it contains a high amount of polyunsaturated fatty acids [7].

Currently, no effective drug for alleviating testicular ischemia-reperfusion injury is clinically available. *Scutellaria baicalensis* Georgi, also called Huangqin in China, is a perennial herb from the Lamiaceae family [8, 9]. It is indigenous to North China, Japan, North Korea, Mongolia, and Russia’s Eastern Siberia and is widely cultivated in many European countries [9]. In China, *Scutellaria baicalensis* Georgi is a common Chinese medicinal herb and has been widely used for more than 2000 years to treat various diseases, such as diarrhea, dysentery, hypertension, hemorrhaging, insomnia, inflammation, and respiratory infections [9, 10]. Nowadays, it is officially listed in Chinese Pharmacopoeia, European Pharmacopoeia, and British Pharmacopoeia [9]. Baicalein (5,6,7-trihydroxyflavone) is a main bioactive constituent derived from the dried root of *Scutellaria baicalensis* Georgi [11]. Its molecular formula and molecular weight are 

\[ C_{15}H_{10}O_5 \] and 270.24, respectively [12]. Various studies have shown that baicalein possesses numerous bioactivities, including antioxidative, anti-inflammatory, antibacterial, antiviral, anticancer, antithrombotic, anxiolytic, antidepressant, and antibacterial functions [13–21]. Furthermore, baicalein has been reported to alleviate ischemia-reperfusion injury in the brain, liver, heart, kidney, spinal cord, and retina [22–27]. Nevertheless, whether or not baicalein mitigates testicular ischemia-reperfusion injury was not documented. Hence, in the present research, the effect of baicalein on ischemia-reperfusion injury induced by rat testicular torsion-detorsion was checked.

2. Materials and Methods

2.1. Laboratory Animals. In this study, male Sprague-Dawley rats were supplied by SLAC Laboratory Animal Co., Ltd., Shanghai City, China. A total of eighty male Sprague-Dawley rats (weight range of 250-300g, 8 weeks old) were used for the experiment. They were kept in a standard animal house where humidity of 55% ± 5%, photoperiod of 12-hour light-dark cycles, and temperature of 21°C±1°C were maintained. Rats could unrestrainedly obtain sterilized pellet diet and autoclaved water. Before surgical procedure, rats stayed in the animal house for 7 days to adapt to the conditions. The institutional animal ethics committee of our university approved the experimental protocol. All animal procedures were conducted in the light of regulations of laboratory animal care and use.

2.2. Testicular Ischemia-Reperfusion Model. According to the study design, experimental animals were randomized into 4 groups \( n = 20 \) in each group: control, testicular ischemia-reperfusion, testicular ischemia-reperfusion-vehicle injection, and testicular ischemia-reperfusion+baicalein therapy groups. Before surgical operation, ketamine (50 mg/kg; Sigma Chemical Company, St. Louis, MO, USA) was administered intraperitoneally to induce anesthesia. After clipping the hairs on the left-sided ilioinguinal region, the region was disinfected with 10% povidone-iodine solution. Subsequently, a left-sided ilioinguinal incision was performed, through which the left testis was pulled out. In the control group, we placed anatraumatic 11-0 suture through the tunica albuginea. Then, the left testis was returned to scrotal position without any other treatment, and the incision was stitched by a 4-0 nonabsorbable silk suture. The performance of testicular ischemia-reperfusion model was as follows. In order to induce testicular ischemia, a 720-degree rotation in a counterclockwise direction was performed on the left testis. To maintain the left testis in this state for 2 hours, the tunica albuginea of the testis and inner wall of the scrotum were stitched together with anatraumatic 11-0 suture. At the end of the testicular ischemic period, we removed the suture and restored the twisted testis to its original position for reperfusion. Baicalein (Sigma Chemical Company) was dissolved in a solution of dimethyl sulfoxide and promptly diluted to the final concentration of 10 mg/ml with normal saline before injection. The final concentration for dimethyl sulfoxide in the injected solution was 10% \((v/v)\). At onset of reperfusion, rats in the testicular ischemia-reperfusion+baicalein therapy group received intraperitoneal injection of baicalein (100 mg/kg) dissolved in a solution of dimethyl sulfoxide and normal saline. The dosage of baicalein used in the current study was based on the results of several previous experimental researches [22, 26, 28–32]. Rats in the testicular ischemia-reperfusion+vehicle injection group underwent intraperitoneal injection of an equal volume of the vehicle (a solution of dimethyl sulfoxide and normal saline) at onset of reperfusion. Lastly, the reperfused testis was replaced into the scrotum, and the incision was stitched. As mentioned above, a total of 80 male rats were randomized into 4 groups \( n = 20 \) in each group. After 4 hours of reperfusion, bilateral orchietomy was performed on 10 rats in each group to examine tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) levels, myeloperoxidase activity, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase protein expression, and malondialdehyde content. Afterwards, these rats were euthanized via inhaling carbon dioxide. After 3 months of reperfusion, bilateral orchietomy was performed on the remaining 10 rats in each group to measure testicular spermatogenesis.

2.3. Measurement of TNF-α and IL-1β Levels by Enzyme-Linked Immunosorbtent Assay (ELISA). Testicular sample (100 mg) was homogenized in ice-cold cell extraction buffer (Thermo Fisher Scientific, Waltham, MA, USA) with protease inhibitors (Sigma Chemical Company). After homogenization, the homogenate was centrifuged at 14,000 × gravity at 4°C for 15 minutes to obtain supernatant. The TNF-α and IL-1β levels in the supernatant were determined by using commercially available ELISA kits (PeproTech, Cranbury, NJ, USA) according to the manufacturer’s instructions. The results of TNF-α and IL-1β levels were expressed as pg/mg tissue.
2.4. Determination of Myeloperoxidase Activity in the Testis. The tissue sample was placed in 50 mM potassium phosphate buffer, homogenized for 10 minutes, and centrifuged at 40,000 × gravity for 30 minutes. The pellet was obtained and suspended in 50 mM potassium phosphate buffer complemented with 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical Company). The suspension was sonicated for 10 seconds and then subjected to 3 freeze-thaw cycles, after which the sonication was performed again for 10 seconds. Subsequently, the suspension was centrifuged again at 40,000 × gravity for 30 minutes, and supernatant was retained. Supernatant (100 μl) was added to the reaction mixture containing 0.167 mg/ml O-dianisidine dihydrochloride (Sigma Chemical Company) and 0.0005% hydrogen peroxide. The change in absorbance per minute in each sample was measured at 460 nm by the use of a spectrophotometer. One unit activity of myeloperoxidase is defined as a quantity inducing degradation of 1 mM of peroxide per minute. Results were shown as U/g tissue.

2.5. Immunoblot for NADPH Oxidase. To detect NADPH oxidase protein expression, we homogenized testicular tissue in cold lysis solution (consisting of 50 mM Tris HCl, pH 7.4, 0.5% sodium deoxycholate, 1 mM dithiothreitol, 0.5 mM ethylenediaminetetraacetic acid, 0.5 μg/ml leupeptin, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, 150 mM NaCl, 2 mM sodium orthovanadate, and 1% nonidet P-40) to produce 10% (w/v) homogenate. Then, the homogenate was placed on ice for 30 minutes, and the supernatant was subsequently collected by centrifugation (14,000 g, 4°C) for 15 minutes. The protein concentration of each sample was measured by a Bradford assay kit (Bio-Rad Laboratories, Hercules, CA, USA). The protein sample was added to a loading buffer and heated at 100°C for 3 minutes. Equivalent protein samples (30 μg/well) were electrophoresed on sodium dodecyl sulfate-polyacrylamide gel and then transferred electrophoretically to nitrocellulose membrane. The membrane was incubated in Tris-buffered saline containing 0.1% Tween-20 and 5% nonfat dry milk at room temperature for 1 hour to block nonspecific binding sites. Next, the membrane was probed overnight at 4°C with primary antibodies for anti-NADPH oxidase subunit gp91-phox (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-β-actin (inner control; Sigma Chemical Company). After being rinsed three times with Tris-buffered saline containing 0.1% Tween-20, the membrane was probed for 1 hour at room temperature with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). Finally, the membrane was washed again three times in Tris-buffered saline containing 0.1% Tween-20, and antibody-reactive protein bands on the membrane were detected with enhanced chemiluminescence developer (Santa Cruz Biotechnology). A GS-700 imaging densitometer (Bio-Rad Laboratories) was used to analyze the intensity of NADPH oxidase and β-actin protein bands. The intensity ratio of NADPH oxidase band to internal reference β-actin band from the same sample expressed a relative level of NADPH oxidase protein expression.

2.6. Assessment of Testicular Malondialdehyde Concentration. Approximately 100 mg of testicular tissue sample was immersed in 1 ml chilled malondialdehyde lysis buffer. A homogenizer was used to homogenize the testicular tissue. The resulted suspension was centrifuged (5000 g, 15 minutes, 4°C), and the obtained supernatant was used for malondialdehyde analysis. Protein level of the supernatant was assessed using the method described by Bradford [33]. Testicular malondialdehyde concentration was determined using thiobarbituric acid reactive substance assay kit (Nanjing Jiancheng Institute of Bioengineering, Nanjing City, China) according to the method of Ohkawa et al. [34]. Results were shown as nmol/mg protein.

2.7. Spermatogenic Evaluation. Testicular spermatogenesis was evaluated by some markers, including testicular weight, seminiferous tubular diameter, number of germ cell layers, and Johnsen’s score. Briefly, testicular specimen was weighed and placed into Bouin’s solution for fixation. Thereafter, the sample was passed through the increased ethanol series and embedded in paraffin wax. The paraffin slice with 5 μm thickness was obtained using a microtome. Section was deparaffinized, and then staining of section was performed using hematoxylin and eosin solutions (Sigma Chemical Company). Twenty round seminiferous tubules in each testicular section were inspected by a skilled pathologist in a blinded manner with the help of a light microscope. An eyepiece micrometer was installed on an optical microscope and used to determine seminiferous tubular diameter. Next, the number of germinal cell layers in the seminiferous tubule was assessed by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen. Moreover, the germinal epithelial cell maturity in the seminiferous tubule was evaluated by counting the epithelial cell layer’s number from basement membrane to tubular lumen.

2.8. Statistical Methods. Comparative analyses were conducted using the GraphPad Prism statistical software package, Version 4.0 (GraphPad Software Inc., San Diego, CA, USA). The values of all variables were shown as mean ± standard deviation. The Shapiro-Wilk test was used to confirm normal distribution of data. The differences in the variables between ipsilateral and contralateral testes within group were analyzed by a two-tailed Student t-test. One-way analysis of variance followed by Student-Newman-Keuls post hoc test was utilized to identify the differences in the data among four groups. The differences with P values less than 0.05 were regarded as statistically significant.

3. Results

3.1. TNF-α and IL-1β Levels in Testicular Tissue. The results of TNF-α and IL-1β levels in the testes in control, testicular ischemia-reperfusion, testicular ischemia-reperfusion + vehicle injection, and testicular ischemia-reperfusion + baikalein therapy groups are shown in Figure 1. Compared with the
ences were seen in the TNF-α levels in ipsilateral torsional testes among four groups (P < 0.05). Treatment with baicalein caused a significantly reduced level (P < 0.05). However, baicalein treatment could significantly reduce myeloperoxidase activity of the ipsilateral testes in comparison with that in the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups (P < 0.05). There was no significant difference in myeloperoxidase activity of contralateral nontorsional testes among four groups (P > 0.05).

3.2. Myeloperoxidase Activity in the Testis. As shown in Figure 2, the myeloperoxidase activity in ipsilateral torsional testes in the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups was significantly higher compared with that in the control group (P < 0.05). However, baicalein treatment could significantly reduce myeloperoxidase activity of the ipsilateral testes in comparison with that in the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups (P < 0.05).

3.3. Testicular NADPH Oxidase Protein Expression. As shown in Figure 3, both testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle injection significantly upregulated the NADPH oxidase protein expression in ipsilateral torsional testes in comparison with the control group (P < 0.05). Baicalein treatment significantly reduced the elevation of NADPH oxidase protein expression in ipsilateral testes triggered by both testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle injection (P < 0.05). However, the NADPH oxidase protein expression in contralateral nontorsional testes was not considerably different among four groups (P > 0.05).

3.4. Testicular Concentration of Malondialdehyde. As shown in Figure 4, the malondialdehyde concentration in ipsilateral torsional testes was increased significantly following both testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle injection in contrast to the control group (P < 0.05). After baicalein administration, the malondialdehyde concentration in ipsilateral testes was decreased significantly when compared with the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle injection.
3.5. Testicular Spermatogenic Findings. As shown in Figures 5 and 6, testicular weight, seminiferous tubular diameter, number of germ cell layers, and Johnsen’s score were significantly lower in ipsilateral torsional testes in the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups than the control group (P < 0.05). In the baicalein-treated group, it was observed that the four parameters in ipsilateral testes had significantly improved compared with the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups (P < 0.05). In contrast, the differences in the four parameters of contralateral nontorsional testes were not significant among four groups (P > 0.05).

4. Discussion

Testicular torsion, also called spermatic cord torsion, is one of the most critical emergencies in the department of urology. Its annual incidence is 1 in 4000 males between 1 and 25 years of age [36]. Urgent surgical detorsion is an essential therapeutic approach in order to avoid testicular torsion-induced infarction. Survival will be achieved in 90%-100% of the affected testes if surgical detorsion is performed within 6 hours of the onset of acute testicular pain [37]. Testicular atrophy rates remain between 9.1% and 73.3% despite early surgical detorsion [1, 2]. In our study, 2-hour left testicular torsion, along with 3-month detorsion, caused damage to spermatogenesis in ipsilateral testes, which was reflected by significant decreases in testicular weight, seminiferous tubular diameter, number of germ cell layers, and Johnsen’s score (Figures 5 and 6). Testicular damage that occurs during testicular torsion-detorsion is called as ischemia-reperfusion injury.
Ischemia-reperfusion injury is related to overgeneration of reactive oxygen species [3–5]. Increased reactive oxygen species have deleterious effects on cellular components by causing lipid peroxidation, structural alteration of protein, and DNA oxidation [6]. Owing to extremely reactive nature and short life of reactive oxygen species, their measurement is very difficult [38]. Reactive oxygen species have a peroxidative effect on cellular membrane lipids [6]. The final product of lipid peroxidation is malondialdehyde [38]. Hence, malondialdehyde is considered as a common marker of reactive oxygen species [38]. Our results revealed an increased malondialdehyde level and reduced spermatogenesis in ipsilateral testes of the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups, compared to the control group (Figures 4–6). These findings demonstrate that the production of excessive reactive oxygen species induces the loss of spermatogenesis during testicular ischemia-reperfusion. However, there were no significant differences in malondialdehyde level and spermatogenesis of ipsilateral testes between the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups (Figures 4–6). These data suggest that the vehicle has no effect on testicular ischemia-reperfusion injury. We also observed that malondialdehyde level was significantly decreased, while spermatogenesis was significantly increased in ipsilateral testes of the baicalein-treated group when compared to the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups (Figures 4–6). These results imply that baicalein exerts protective effect against testicular ischemia-reperfusion injury via decreasing the level of reactive oxygen species. Moreover, the clinical application of baicalein aluminum capsule has been found to be safe and efficacious in the treatment of enteritis and dysentery since it was put on the market in 1976, with effective rates of up to 97.27% for acute and chronic enteritis and 93% for bacillary dysentery [39]. Taken together, these data may provide a rationale for the clinical application of baicalein to treat testicular ischemia-reperfusion injury. Nevertheless, the mechanisms associated with baicalein-mediated reduction of reactive oxygen species are still unclear.

Inflammation is considered to contribute substantially to testicular ischemia-reperfusion injury [40]. During testicular ischemia-reperfusion, germ cells and interstitial macrophages in testis produce a large number of proinflammatory cytokines, such as TNF-α and IL-1β [41]. These cytokines induce chemotactic movement of neutrophils into ischemia-reperfusion testis [41]. A major source of reactive oxygen species overgeneration during tissular ischemia-reperfusion is neutrophils that infiltrate into ischemia-reperfusion tissue [42, 43]. NADPH oxidase in neutrophils plays an important role in reactive oxygen species generation [42, 44]. Calcium influx into neutrophils during ischemia

![Figure 5: Effects of testicular ischemia-reperfusion (IR), testicular IR+vehicle (Ve) injection, and baicalein (Ba) treatment on (a) testicular weight, (b) seminiferous tubular diameter, (c) number of germ cell layers, and (d) Johnsen’s score in rat testicular tissue. Grey columns: ipsilateral testes; white columns: contralateral testes. Values are mean ± standard deviation (n = 10/group). *Statistically different from the control group (P < 0.05), †statistically different from contralateral testes in the same group (P < 0.05), and §statistically different from ipsilateral testes in the IR and IR+Ve groups (P < 0.05).]
activates NADPH oxidase [45]. During the reperfusion period, oxygen is reintroduced into ischemic tissue. Activated NADPH oxidase catalyzes the conversion of oxygen into superoxide anion [42]. The reaction of superoxide anion with itself produces hydrogen peroxide [46]. Superoxide anion reacts with hydrogen peroxide to generate hydroxyl radical [47]. Consequently, increased reactive oxygen species are formed in the process of ischemia-reperfusion. These increased reactive oxygen species are a reason for cellular injury [6]. Myeloperoxidase is an enzyme found primarily in azurophilic granules of neutrophils [48]. The enzyme activity reflects the number of neutrophils in the inflamed tissue [48]. Hence, myeloperoxidase activity serves as an index of neutrophil infiltration in tissue [48]. The glycoprotein gp91-phox is an essential component of the NADPH oxidase in neutrophils [49, 50]. For this reason, we used anti-NADPH oxidase subunit gp91-phox antibody to detect NADPH oxidase protein expression. In the present study, both testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle injection caused significant increases in TNF-α and IL-1β levels, myeloperoxidase activity (an index of neutrophil infiltration in tissue), NADPH oxidase protein expression, and malondialdehyde content (a reliable index of reactive oxygen species) and resulted in a significant decrease in spermatogenesis in ipsilateral testes (Figures 1–6). These findings imply that testicular ischemia-reperfusion leads to excessive secretion of TNF-α and IL-1β, increased neutrophil recruitment into the testis, upregulation of NADPH oxidase expression in neutrophils, and reactive oxygen species overproduction, which can cause spermatogenic injury. Furthermore, our study indicated that baicalein treatment significantly reduced TNF-α and IL-1β levels, myeloperoxidase activity, NADPH oxidase expression, and malondialdehyde content and significantly improved spermatogenesis in ipsilateral testes (Figures 1–6). These data suggest that baicalein protects testicular spermatogenesis through the inhibition of TNF-α and IL-1β secretion, neutrophil recruitment into the testis, and NADPH oxidase protein expression in neutrophils to decrease reactive oxygen species production.

Baicalein at a dose of 100 mg/kg showed effective therapeutic effects on ischemia-reperfusion injury in murine liver,
brain, and spinal cord [22, 26, 28–32]. Thus, we selected the dose in a murine model of testicular ischemia-reperfusion injury. In the current research, baicalein was only injected once at a single dose of 100 mg/kg body weight. Baicalein administration ameliorated histopathological damage in ipsilateral testes and recovered spermatogenesis to near normal (Figures 5 and 6). We did not investigate the therapeutic effect of baicalein at different administration doses or administration frequencies in this study. Hence, additional research is required to determine the most effective dose and frequency of baicalein administration.

The condition of the contralateral untwisted testis is debatable after unilateral testicular ischemia-reperfusion. In some studies, it has been reported that unilateral testicular ischemia-reperfusion induces harmful effect on the contralateral testis [51, 52]. In contrast, other studies have indicated that no injury is observed in the contralateral testis [53, 54]. Our results revealed that TNF-α and IL-1β levels, myeloperoxidase activity, NADPH oxidase expression, malondialdehyde content, and spermatogenesis of contralateral testes in the testicular ischemia-reperfusion group were not statistically different from those in the control group (Figures 1–6). These findings suggest that unilateral testicular ischemia-reperfusion does not affect the contralateral testis.

The testicular ischemia-reperfusion+vehicle injection group played an important role in our study. The results of comparison in spermatogenesis of ipsilateral testes between the testicular ischemia-reperfusion and testicular ischemia-reperfusion+vehicle groups showed that the vehicle had no effect on testicular ischemia-reperfusion injury (Figures 5 and 6). In addition, the results of comparison in spermatogenesis of ipsilateral testes between the testicular ischemia-reperfusion+baicalein dissolved in the vehicle and testicular ischemia-reperfusion+vehicle groups indicated that baicalein attenuated testicular ischemia-reperfusion injury (Figures 5 and 6). Consequently, the testicular ischemia-reperfusion+vehicle group is necessary to the present study.

Our study revealed that unilateral testicular ischemia-reperfusion led to ipsilateral testicular atrophy, which was characterized by reduced testicular weight and atrophic seminiferous tubules with reduced seminiferous tubular diameter, disrupted germinal cell layers, spermatogenic arrest, and absence of spermatooza (Figures 5 and 6). The Masson trichrome-stained testicular section is often used to quantify collagen deposition as an index of fibrosis [55]. Some investigators used Masson trichrome-stained testicular section to find that testicular atrophy after testicular ischemia-reperfusion was usually accompanied by testicular fibrosis [55–58]. In a later study, we will try to use Masson trichrome-stained testicular section to show fibrosis in atrophic testes.

5. Conclusion

Our study is the first to indicate that baicalein exerts a protective effect against ischemia/reperfusion-induced testicular injury via inhibiting TNF-α and IL-1β secretion, neutrophil infiltration in the testis, and NADPH oxidase protein expression in neutrophils to reduce reactive oxygen species production. These results suggest that baicalein may be an effective therapeutic agent for patients with testicular torsion-detorsion. However, human trials should be conducted to validate the clinical benefit of baicalein.

Data Availability

All data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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