Int J Clin Exp Med 2017;10(3):5125-5133
www.ijcem.com /ISSN:1940-5901/IJCEM0024510

Original Article

New wenshen shengjing decoction protects mouse testis from oxidative damages caused by cyclosporine A

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Received January 20, 2016; Accepted June 8, 2016; Epub March 15, 2017; Published March 30, 2017

Abstract: Aims: The present study aimed to investigate the protective effect of new Wenshen Shengjing Decoction (WSSJD) in alleviating oxidative damages in testis tissues caused by cyclosporine A (CsA), as well as its mechanism of action. Methods: A total of 90 Kunming male mice of 8 weeks old and 35-40 g body weight were divided randomly into six groups of 15 mice, including control group, vehicle (DMSO) group, model (CsA) group, western medicine (CC) group, WSSJD group and new WSSJD group. Hematoxylin and eosin staining was performed to investigate the development of seminiferous tubules in the testes of mice. To examine the antioxidant capacity of mouse testes, enzyme-linked immunosorbent assay was employed to measure malondialdehyde, glutathione, glutathione peroxidase and catalase activity levels in testis tissues. To detect the apoptosis of spermatogenic cells, testis tissue slices embedded in paraffin were subjected to terminal dexynucleotidyl transferase-mediated dUTP nick end labeling assay. Flow cytometry was carried out to examine mitochondrial activity and ROS content, and the apoptosis, survival and death of mature sperms from mouse epididymis. Results: Both WSSJD and new WSSJD alleviated damages in the development of seminiferous tubules induced by CsA, and enhanced the development capability of spermatogenic cells. New WSSJD had excellent antioxidative effects on damaged testis tissues. It could significantly reduce the number of apoptotic spermatogenic cells induced by superoxides in mouse testis. In the meanwhile, it enhanced mitochondrial activity and reduced ROS levels in sperms from mouse epididymis. It highly increased the survival rate of mature sperms in mouse epididymis. Conclusions: The present study demonstrates that new WSSJD protects the spermatogenesis function of testis in mouse from oxidative damages caused by CsA, possibly by elevating antioxidative levels and reducing the apoptosis and death of spermatogenic cells and sperms.

Keywords: New wenshen shengjing decoction, cyclosporine A, testis, oxidative damage, apoptosis

Introduction

The technology of organ transplantation has been developed rapidly in recent years, such as liver [1], kidney [2], heart and lung [3] transplantations. After organ transplantation, immunosuppressive agents are usually required to carry out a long-term immunosuppression process to avoid immune rejection. Cyclosporine A (CsA) is a kind of fungal polypeptide that can inhibit the production of lymphokines and the signaling pathway mediated by T cell receptors. As an immunosuppressive agent, CsA is widely used in the immunosuppression after organ transplantation or the treatment of autoimmune diseases [4]. However, immunosuppression is a long process, and long-term use of CsA produces a series of toxic side effects, such as dysfunction of blood vessels [5], hepatotoxicity [6], ovarian injury [7], and testicular and sperm toxicity [8], which limit the clinical application of CsA.

Testis is the place for spermatogenesis, and destruction of testicular structure and function can directly result in disordered spermatogenesis. It is reported that reactive oxygen species (ROS) damages the structure of male and female germ cells, leading to their dysfunction [9]. A study shows that the toxic side effects of

CsA on kidney, liver, heart, ovary and testis are caused by oxidative stress [10]. Excessive ROS production in testis leads to cell membrane lipid peroxidation, damaged DNA, apoptotic spermatogenic cells, reduced sperm activity and motility. Long-term treatment with CsA damages the antioxidant system, reduces the contents of glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase (CAT) [11], and increases the content of malondialdehyde (MDA) in the testis, resulting in severely disturbed testis tissue development and spermatogenesis. Therefore, use of antioxidant drugs may alleviate oxidative damages in testis tissues caused by CsA.

Currently, ellagic acid [11], lycopene [12], N-acetylcysteine [13], black grape extract [14], and taurine [15] have been used as external antioxidants on animals to reduce the toxic side effects of CsA on different organs. It is demonstrated that the antioxidative effect of single drug is weaker than that of composite drugs [16]. Wenshen Shengjing Decoction (WSSJD) is a kind of composite Chinese traditional medicine that has excellent effect in the clinical treatment of dyszoospermia. It is mainly composed of *Cornu Cervi Nippon Parvum*, *Panax ginseng*, *Cynomorium songaricum*, *Cistanche deserticola*, *Radix Astragali*, *Epimedium brevicornum* and *Angelica sinensis* [17], all of which have significant antioxidative effects. Antler extract has antagonistic action on lipid peroxidation caused by O_2^- and OH free radicals [18]. Ginsenoside Rg1 is able to scavenge excessive intracellular ROS, and inhibits cell apoptosis [19]. Polyphenol, polysaccharide anthocyanins and triterpenes in cynomorium songaricum could significantly reduce peroxidation incidence in cells [20]. In addition, polysaccharides in Astragalus mongholicus [21], and flavonoids and polysaccharides in epimedium brevicornum [22] have excellent inhibitory effects on lipid peroxidation, and can capture superoxide radical, hydroxy radical and DPPH free radical. Previously, we have reported that WSSJD significantly alleviates oxidative damages in testis tissues caused by another immunosuppressive agent, cyclophosphamide, and reduces the apoptosis of spermatogenic cells [17]. In the present study, in order to improve its antioxidative function, we alter the composition of WSSJD, and test the effect of new WSSJD in alleviating oxidative damages in testis tissues caused by CsA, as well as its effect on the apoptosis of spermatogenic cells and sperms.

Materials and methods

Animals

A total of 90 Kunming male mice of 8 weeks old with 35-40 g body weight were raised in cages individually under 21 ± 3°C and 55-65% humidity with free access to food and water. The mice were purchased from Changchun Institute of Biological Products Co., Ltd. (Changchun, Jilin, China) under the license of SCXK (Jilin) 2006-0001. The mice were divided randomly into six groups of 15 mice, including control group, solvent (DMSO) group, model (CsA) group, western medicine (CC) group, Wenshen Shengjing Decoction (WSSJD) group and new Wenshen Shengjing Decoction (WSSJD) group. Mice in CsA group, CC group, WSSJD group and new WSSJD group received intraperitoneal injection of 15 mg/kg CsA (Powerdone Pharmaceutics Co., Ltd., Datong, China)·per day [23] for consecutive 30 days. Mice in control group received the same amount of saline, while mice in DMSO group received the same amount of DMSO solvent. In the meantime, mice in CC group were lavaged with 21.6 mg/kg clomifene citrate (GKH Pharmaceutical Ltd., Guangzhou, China) per day [24], and mice in WSSJD group and new WSSJD group were lavaged with 12 g crude drug/kg/day of these drugs (*Cornu Cervi Nippon Parvum*, *Panax ginseng*, *Cynomorium songaricum*, *Cistanche deserticola*, *Radix Astragali*, *Epimedium brevicornum* and *Angelica sinensis*; Jilin Kangnaixin Pharmacy, Jilin, China) respectively per day, and new Wenshen Shengjing Decoction (compared with Wenshen Shengjing Decoction, the contents of *Panax ginseng*, *Cynomorium songaricum*, *Radix Astragali* and *Epimedium brevicornum* were adjusted accordingly). The mice in control group and CsA group were lavaged with the same amount of saline for 30 days. All animal experiments were conducted according to the ethical guidelines of Jilin Medical University.

Hematoxylin and eosin (HE) staining

Male mouse testis was fixed with 4% paraformaldehyde, and subjected to gradient dehydration using alcohol, followed by xylene transparency. After being paraffin-embedded, the samples were prepared into 5 μm slices. After staining with HE, the testis seminiferous epithelia were observed under a microscope.

Spectrophotometer colorimetric assay

The testis was homogenized in saline on ice, and then centrifuged at 3000 rpm for 15 min. The supernatants were used to measure the contents of MDA and GSH, and the enzyme activity of GSH-Px and CAT in testis tissues using spectrophotometer colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Terminal dexynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

Testis tissue slices embedded in paraffin were subjected to TUNEL assay (Boster, Wuhan, China) for the detection of apoptosis of spermatogenic cells. Cells with brown granules in nuclei were identified as positive cells with apoptosis. Five slices were tested for each animal, and ten seminiferous tubules were randomly chosen from each slice. The number of apoptotic spermatogenic cells in each seminiferous tubule was counted.

Flow cytometry

Cauda epididymidis were harvested after the mice were sacrificed, and cut into pieces before incubation in 2 ml phosphate-buffered saline at 37°C to obtain epididymis suspensions. The suspensions were then incubated at 37°C for 10 min to allow sperms to swim out. Sperm conglomerations were discarded and the sperm samples were resuspended into the concentration of 5×10^6/ml.

To detect mitochondrial activity of epididymis sperms, Rh123 (Sigma-Aldrich, St. Louis, MO, USA) was added to a final concentration of 5 μg/mL in semen samples, and the samples were incubated for 5 min at 37°C in the dark. The sperms were then washed with dye-free phosphate-buffered saline to eliminate non-specific binding of dye to the mitochondria, followed by centrifugation. Finally, the samples were resuspended in phosphate-buffered saline and immediately examined by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA). The average fluorescence intensity of Rh123 in each semen sample was examined and recorded.

To test the content of reactive oxygen species (ROS), 0.5 ml sperm suspension was mixed with 5 μL DCFH-DA (1 mmol/L; Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37°C in dark for 15 min. The samples were washed with 5 ml phosphate-buffered saline twice. The average fluorescence intensity of ROS in each group was measured using flow cytometry.

To determine the apoptosis and death rates of epididymis sperms, Annexin V-propidium iodide (PI) double staining was used (BD Biosciences, San Jose, CA, USA). Sperm suspension was adjusted to the density of $2\text{-}3\times10^5$ sperms/ml, and digested using 2 μl trypsin for 5 min. Then, the density of sperm suspension was adjusted to $1\text{-}2\times10^5$ sperms/ml. After that, 195 μl sperm suspension was mixed with 5 μl Annexin V-fluorescein isothiocyanate, followed by incubation at room temperature for 10 min. Following the addition of 10 μl PI, the samples were incubated at room temperature in dark for 10-15 min. The rates of normal sperms (low staining with both Annexin V and PI), apoptotic sperms (high staining with Annexin V and low staining with PI) and dead sperms (high staining with both Annexin V and PI) were measured using flow cytometry.

Statistical analysis

The results were analyzed using SPSS 13.0 software (IBM, Armonk, NY, USA). The data were expressed as means ± standard deviations. Differences between groups were evaluated for significance using one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

Both WSSJD and new WSSJD alleviate damages in the development of seminiferous tubules induced by CsA

To test the effect of new WSSJD on the development of seminiferous tubules in the testis of mouse, HE staining was performed. Under the microscope, the spermatogenic cells in control, DMSO, WSSJD and new WSSJD groups were tightly arranged. Their seminiferous tubules had intact edges, no atrophy or collapse, and abundant sperms inside the tubule lumen, with many seminiferous epithelium layers (**Figure 1A-D**). By contrast, seminiferous tubules in CsA group and CC group showed collapsed edges, reduced diameters, decreased numbers of seminiferous epithelium layers, disordered

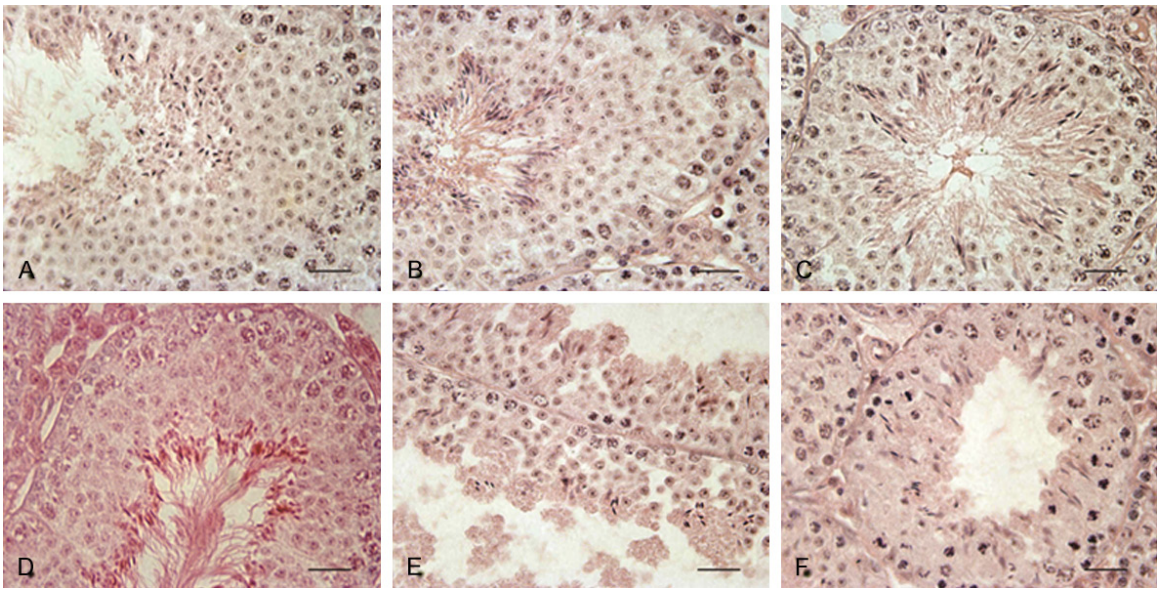

Figure 1. Effect of new WSSJD on the development of seminiferous tubules in the testes of mice. Hematoxylin and eosin staining of mice testes tissues from (A) control group, (B) DMSO group, (C) WSSJD group, (D) new WSSJD group, (E) CsA group, and (F) CC group. DMSO, dimethyl sulphoxide; CsA, cyclosporine A; CC, western medicine; WSSJD, Wenshen Shengjing Decoction. Scale bar, 50 μm.

Table 1. Levels of MDA and GSH and activities of GSH-Px and CAT in testis tissues of mice

Groups	MDA (nmol/mg protein)	GSH (mg/g protein)	GSH-Px activity (U)	CAT activity (U/mg protein)
Control group	1.44 ± 0.06	14.54 ± 0.71	55.10 ± 2.27	6.50 ± 0.11
DMSO group	1.47 ± 0.06	14.43 ± 0.54	53.21 ± 2.06	6.47 ± 0.13
CsA group	5.89 ± 0.23*	5.63 ± 0.28*	32.60 ± 3.55*	3.46 ± 0.16*
CC group	5.64 ± 0.24*	6.39 ± 0.24*	37.41 ± 1.97*	4.18 ± 0.17$^{*,\#}$
WSSJD group	2.65 ± 0.45$^{*,\#,\Delta}$	8.16 ± 0.41$^{*,\#,\Delta}$	42.65 ± 1.16$^{*,\#}$	4.46 ± 0.18$^{*,\#}$
New WSSJD group	2.14 ± 0.38$^{\#,\Delta}$	9.39 ± 0.36$^{*,\#,\Delta}$	46.15 ± 2.80$^{*,\#,\Delta}$	5.55 ± 0.17$^{*,\#,\Delta,\blacktriangle}$

Note: MDA, malondialdehyde; GSH, glutathione; GSH-Px, glutathione peroxidase; CAT, catalase. *, $P < 0.05$ compared with control group; $^{\#}$, $P < 0.05$ compared with CsA group; $^{\Delta}$, $P < 0.05$ compared with CC group; $^{\blacktriangle}$, $P < 0.05$ compared with WSSJD group. DMSO, dimethyl sulphoxide; CsA, cyclosporine A; CC, western medicine; WSSJD, Wenshen Shengjing Decoction.

spermatogenic cell arrangements, enlarged tubule lumens, and few sperms inside the tubule lumen (**Figure 1E** and **1F**). The result suggests that both WSSJD and new WSSJD alleviate damages in the development of seminiferous tubules induced by CsA.

New WSSJD has excellent antioxidative effects on damaged testis tissues

To measure the levels of MDA and GSH and the activities of GSH-Px and CAT in mice testis tissues, spectrophotometer colorimetric assay was employed. The data showed that the levels of MDA and GSH and the activities of GSH-Px and CAT in DMSO group were not significantly different from those in control group. In CsA group, MDA level was significantly increased, and the level of GSH and the activities of GSH-Px and CAT were significantly decreased compared with control group ($P < 0.05$). In WSSJD and new WSSJD groups, MDA level was significantly reduced while GSH level and the activities of GSH-Px and CAT were significantly enhanced compared with CsA group ($P < 0.05$). In CC group, CAT activity was significantly higher than CsA group ($P < 0.05$), but MDA and GSH levels and GSH-Px activity were not significantly different from that in CsA group ($P > 0.05$). Furthermore, new WSSJD group had significantly reduced MDA level, and significantly enhanced GSH level and GSH-Px and CAT activities compared with CC group ($P < 0.05$). Of note, the effect of new WSSJD in increasing CAT

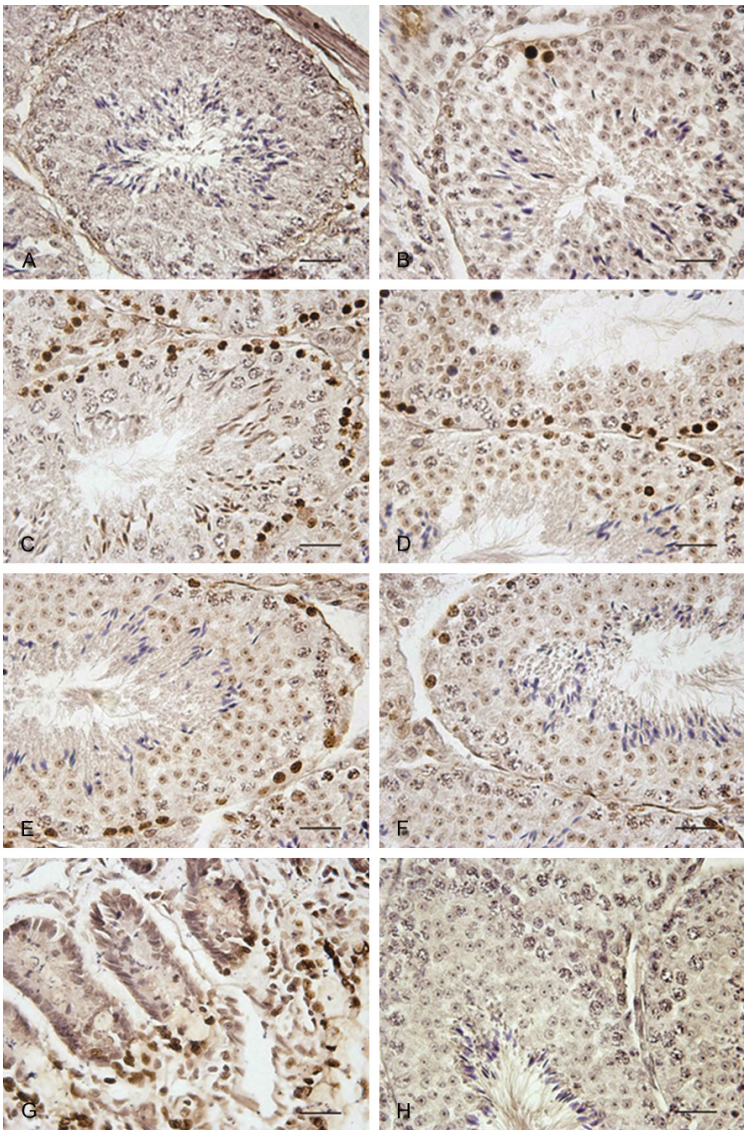

Figure 2. Apoptosis of spermatogenic cells in mice testes tissues. TUNEL assay of (A) control group, (B) DMSO group, (C) CsA group, (D) CC group, (E) WSSJD group, (F) new WSSJD group, (G) positive group, and (H) negative group. DMSO, dimethyl sulphoxide; CsA, cyclosporine A; CC, western medicine; WSSJD, Wenshen Shengjing Decoction. Scale bar, 50 μm.

activity was stronger than that of WSSJD (**Table 1**). The results indicate that new WSSJD has excellent antioxidative effects on damaged testis tissues.

New WSSJD has strong effect in reducing the number of apoptotic spermatogenic cells in mice testis

To detect the apoptosis of spermatogenic cells in mice testis tissues, TUNEL was carried out. The data showed that apoptotic spermatogenic cells had brown nuclei, and the apoptotic cells were mainly spermatogonia and primary spermatocytes (**Figure 2**). The number of apoptotic spermatogenic cells in DMSO group was not significantly different from that in control group, but that in CsA group was significantly higher than that in control group ($P < 0.05$). In addition, the numbers of apoptotic spermatogenic cells in CC, WSSJD and new WSSJD groups were significantly lower than that in CsA group ($P < 0.05$). Moreover, the number of apoptotic spermatogenic cells in new WSSJD group was significantly lower than those in CC and WSSJD group ($P < 0.05$) (**Table 2**). The results suggest that new WSSJD has strong effect in reducing the number of apoptotic spermatogenic cells in mice testis.

New WSSJD enhances mitochondrial activity and reduces ROS levels in sperms from mouse epididymis

To examine how new WSSJD affects mitochondrial activity and ROS content in mature sperms from mouse epididymis, we used flow cytometry to measure the fluorescence intensity of RH123 and ROS, respectively. The data showed that mitochondrial activity and ROS content in DMSO group were not significantly different from those in control group ($P > 0.05$), but mitochondrial activity in CsA group was significantly lower and ROS content was significant higher than that in control group ($P < 0.05$). In addition, WSSJD and new WSSJD groups had elevated mitochondrial activity and reduced ROS content compared with CsA group ($P < 0.05$). New WSSJD group showed increased mitochondrial activity and decreased ROS content compared with CC group ($P < 0.05$), but WSSJD group had no significantly different mitochondrial activity compared with CC group ($P > 0.05$). Furthermore, the ROS content in new WSSJD group was significantly lower than that in WSSJD group ($P < 0.05$) (**Table 3**). The

Table 2. The number of apoptotic spermatogenic cells in seminiferous tubules from mice testis

Groups	Number of apoptotic spermatogenic cells/seminiferous tubule
Control group	1.67 ± 0.33
DMSO group	2.67 ± 0.33
CsA group	42.00 ± 2.52*
CC group	32.00 ± 3.61*,#
WSSJD group	20.67 ± 2.33*,#,Δ
New WSSJD group	10.67 ± 3.48*,#,Δ,▲

Note: *, P < 0.05 compared with control group; #, P < 0.05 compared with CsA group; Δ, P < 0.05 compared with CC group; ▲, P < 0.05 compared with WSSJD group. DMSO, dimethyl sulphoxide; CsA, cyclosporine A; CC, western medicine; WSSJD, Wenshen Shengjing Decoction.

Table 3. Average fluorescence intensity of RH123 and ROS in mature sperms from mouse epididymis

Groups	RH123 fluorescence intensity	ROS fluorescence intensity
Control group	40.27 ± 3.12	1.03 ± 0.12
DMSO group	40.17 ± 3.03	1.13 ± 0.03
CsA group	22.77 ± 1.65*	2.13 ± 0.09*
CC group	26.00 ± 0.76*	1.83 ± 0.09*,#
WSSJD group	30.67 ± 1.13*,#	1.47 ± 0.03*,#,Δ
New WSSJD group	34.27 ± 0.85#,Δ	1.20 ± 0.06#,Δ,▲

Note: *, P < 0.05 compared with control group; #, P < 0.05 compared with CsA group; Δ, P < 0.05 compared with CC group; ▲, P < 0.05 compared with WSSJD group. DMSO, dimethyl sulphoxide; CsA, cyclosporine A; CC, western medicine; WSSJD, Wenshen Shengjing Decoction.

results indicate that new WSSJD enhances mitochondrial activity and reduces ROS levels in sperms from mouse epididymis.

New WSSJD is highly effective in increasing the survival rate of mature sperms in mouse epididymis

To investigate the effect of new WSSJD on the apoptosis, survival and death of mature sperms in mouse epididymis, flow cytometry was used. The apoptotic rates were not significantly different among all groups (P > 0.05). In addition, the survival and death rates in DMSO group were not different from those in control group, but the survival rate was significantly decreased and the death rate was significantly enhanced in CsA group compared with control group (P < 0.05). The survival rates were significantly enhanced and the death rates were significantly decreased in both WSSJD and new WSSJD groups compared with CsA group. Moreover, the survival rate in new WSSJD group was significantly higher than those in CC group and WSSJD group (P < 0.05). The death rate in new WSSJD group was significantly lower than that in CC group (P < 0.05), but was not significantly different from that in WSSJD group (P > 0.05) (**Table 4**). The results suggest that new WSSJD is highly effective in increasing the survival rate of mature sperms in mouse epididymis.

Discussion

As an immunosuppressive agent, CsA significantly enhances the survival rate and life quality of patients with organ transplantation [25]. However, the toxic side effects of CsA on various organs of the patients have limited its clinical applications [6, 10]. Of note, the testicular toxicity of CsA has caused declining fertility or infertility in male patients [26]. In our previous study, we find that another immunosuppressive agent, cyclophosphamide, also significantly reduces antioxidase activity in testis tissues and damages the development of seminiferous tubules, finally leading to enhanced apoptosis of spermatogenic cells and sperms [17]. In addition, the study also shows that lowered testosterone levels induced by cyclophosphamide damage male fertility [17]. However, it is not clear whether the damage in testis tissues caused by CsA is related to oxidative stress.

Sperms are a kind of cells with special structures in the body, which are easily damaged by oxidative stress [27]. It is shown that long-term treatment with CsA disturbs the antioxidative system in testis tissues, and reduces the content of GSH, activities of GSH-Px and CAT [11], leading to enhanced levels of MDA. A report shows that the effect of CsA on semen quality is dose-dependent, with higher doses of CsA having more severe damages on male fertility [28]. Misro et al. report that 1 mg/kg CsA added into semen significantly reduces sperm motility in vitro [29]. Turk et al. find that lavage with 15 mg/kg CsA significantly reduces the density and motility of sperms in mouse epididymis, and enhances the percentage of abnormal sperms [11]. In the present study, lavage with 15 mg/kg CsA on healthy male mice for one month significantly reduces the mitochondrial activity, increases the ROS level, decreases sperm survival rate, and enhances sperm

Table 4. Effect of new WSSJD on the apoptosis, survival and death of mature sperms in mouse epididymis

Groups	A1+A2 (%)	A3 (%)	A4 (%)
Control group	1.18 ± 0.50	93.91 ± 0.97	4.91 ± 0.69
DMSO group	2.02 ± 0.42	91.09 ± 0.21	6.89 ± 0.23
CsA group	3.18 ± 0.94	55.28 ± 1.53*	41.54 ± 2.46*
CC group	2.67 ± 1.40	82.23 ± 1.22$^{*,\#}$	15.10 ± 1.12$^{*,\#}$
WSSJD group	4.36 ± 2.16	84.42 ± 1.39$^{*,\#}$	11.22 ± 1.06$^{*,\#}$
New WSSJD group	3.38 ± 0.77	88.72 ± 1.03$^{*,\#,\Delta,\blacktriangle}$	8.08 ± 0.92$^{\#,\Delta}$

Note: *, $P < 0.05$ compared with control group; $^{\#}$, $P < 0.05$ compared with CsA group; $^{\Delta}$, $P < 0.05$ compared with CC group; $^{\blacktriangle}$, $P < 0.05$ compared with WSSJD group. DMSO, dimethyl sulphoxide; CsA, cyclosporine A; CC, western medicine; WSSJD, Wenshen Shengjing Decoction. A1+A2 (%) shows the apoptotic rate of sperms in mouse epididymis; A3 (%) shows the survival rate of sperms in mouse epididymis; A4 (%) shows the death rate of sperms in mouse epididymis.

death rate. Excessive ROS induces the peroxidation of unsaturated fatty acids in sperm plasma membrane, increases the permeability of mitochondrial membrane and lowers mitochondrial activity, finally leading to the death of sperms.

MDA is one of the final products of lipid peroxidation of polyunsaturated fatty acid attacked by free radicals. It causes crosslinking polymerization of proteins or nucleic acids, with relatively high cytotoxicity [30]. GSH is a kind of low molecular scavenger that can scavenge O_2^-, H_2O_2 and LOOH. Insufficiency or depletion of GSH may lead to toxication of organisms caused by chemicals or environmental factors [31]. GSH-Px and CAT are two important enzymes that can catalyze the degradation of H_2O_2 [32]. Therefore, determination of the contents of MDA and GSH and the activities of GSH-Px and CAT may help reflect the antioxidative capability of tissues and the degrees of oxidative damages. In the present study, CsA significantly reduces the level of GSH and the activities of GSH-Px and CAT, and significantly elevates the level of MDA in testis tissues. Changes in the activities of these enzymes have reduced their ability of scavenging ROS, finally leading to lipid peroxidation of cell membrane.

Monteiro et al. report that treatment with CsA causes significantly increased proportion of connective tissues in testis tissues, degeneration of seminiferous epithelia, serious vacuolization in Sertoli cells, accumulation of residual cytoplasm at seminiferous epithelium edge, and expanded gaps between spermatogenic cells [33]. In the present study, treatment with CsA leads to collapsed edges, reduced diameter, decreased number of epithelial cell layers, disordered spermatogenic cell arrangement, enlarged tubule lumen, and few sperms inside the tubule lumen. In addition, treatment with CsA results in severe apoptosis of spermatogenic cells, which are mainly spermatogonia and primary spermatocytes. These damages may be directly or indirectly related with reduced activity of antioxidases and lower antioxidant levels.

Turk et al. find that ellagic acid and lycopene have limited ability in scavenging ROS, and only partially improve semen quality [11, 34]. It is hypothesized that synergy of multiple antioxidant drugs may extensively promote the antioxidative capacity of testis tissues. In the present study, we have investigated the effect of a compound Chinese medicine preparation, new WSSJD, in protecting testis tissues from oxidative damages. Many components of new WSSJD have their antioxidative effects. Antler extract inhibits lipid peroxidation (formation of MDA) induced by NADPH-vitamin C and Fe^{2+}-cysteine system, and O^{2-} production (formation of reduced cytochrome C) caused by xanthine-xanthine oxidase system in the brain, liver and kidney of rats [18]. Ginsenoside Rg1 is able to scavenge excessive intracellular ROS in SHSY-5Y cells, and inhibits cell apoptosis induced by MPP+ [19]. Polyphenol, polysaccharide anthocyanins and triterpenes in *Cynomorium songaricum* have significant antioxidative effects [20]. In addition, polysaccharides in *Astragalus mongholicus* have protective effect against free radical damages [21]. Flavonoids and polysaccharides in *Epimedium brevicornum* have excellent inhibitory effects on lipid peroxidation, and can scavenge superoxide radical, hydroxy radical and DPPH free radical [22]. The synergistic use of these components has good antioxidative effects on the development of testis tissues. In conclusion, new WSSJD protects testis tissues from oxidative damages caused by CsA. It greatly reduces the toxic side effects of CsA on testis tissues. Therefore, new WSSJD is likely to be used to protect testis tis-

sue from the damages induced by CsA in the treatment of immunosuppression after organ transplantation or autoimmune diseases.

Acknowledgements

This work was supported by Key Research Project of Scientific and Technological Development Program of Jilin Province (No. 20140204033YY), Undergraduate Training Programs for Innovation and Entrepreneurship of Jilin Province (Nos. 2014024 and 2015016) and Program for Innovative Research Team (in Science and Technology) in University of Jilin Province.

Disclosure of conflict of interest

None.

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