Comparison of Intraperitoneal and Intraepididymal Quercetin for the Prevention of Testicular Torsion/Detorsion-induced Injury

Kai-Kai Chi, Wen-Hui Zhang, Guang-Ce Wang, Zhu Chen, Wei He, Suo-Gang Wang, Yong Cui, Peng Lu, Xiao-Jiao Wang, and Hui Chen

OBJECTIVE To compare the effects of intraepididymal quercetin (IE-QE) with those of intraperitoneal quercetin (IP-QE) on testicular torsion/detorsion (TD)-induced ischemia/reperfusion (IR) injury of the testes in an experimental rat model.

METHODS Twenty-four rats were divided into 4 groups: sham (S), TD, TD treated with IP-QE, and TD treated with IE-QE. The IP-QE group received 20 mg/kg QE intraperitoneally, whereas the IE-QE group received quercetin (QE) epididymally. After surgically induced TD, sera and testicular tissues were obtained for the analysis of biochemical parameters including glutathione peroxidase (GPx), malondialdehyde, total antioxidant status, total oxidant status, oxidative stress index, histologic changes, and evaluation of germ cell apoptosis.

RESULTS The oxidative stress index and oxidants (malondialdehyde and total oxidant status) were increased with a concomitant decrease in the antioxidants (GPx and total antioxidant status) in the TD group. Severe histopathological damage, indicated by low Johnsen scores and high testicular injury grades, and germ cell apoptosis were found in the TD group compared with the other groups. Rats treated with QE showed significantly less IR injury, with moderately altered biochemical parameters, histopathological damage, and germinal cell apoptosis compared with the TD group. Most importantly, we found no significant differences in the biochemical parameters, histopathological changes, and germinal cell apoptosis between the IP-QE and IE-QE groups.

CONCLUSION IE-QE was comparable to IP-QE in the treatment of testicular TD. Local QE therapy should be considered as a new approach to treating testicular IR injury due to TD. UROLOGY 99: 106–111, 2017.

Testicular torsion is one of the cases of urologic emergency and causes testicular ischemia injury initially, and subsequently leads to reperfusion injury after blood flow is reestablished, which ultimately results in infertility or subfertility.[1,2] After reperfusion, excessive reactive oxygen species (ROS)[3,4] are produced and released, and cause cellular and tissue damage. Until now, pathological mechanisms accounting for testicular ischemia/reperfusion (IR) injury have not been completely clarified. This injury is partially characterized by a state of imbalance between ROS and cellular antioxidative systems.[5-7]

Recent studies have shown the utility of plant-derived antioxidants in protecting the testis against IR injury. Quercetin (QE) has been investigated as one of the therapeutic antioxidants with the potential to decrease testicular injury after IR processes.[8-11] Previous studies of IR injury utilized intraperitoneal (IP) injection of QE almost exclusively. Hence, the current study aimed to evaluate the efficacy of intraperitoneal vs intraepididymal administration of QE in alleviating testicular torsion/detorsion (TD) in animal models. This is the first study to introduce intraepididymal quercetin (IE-QE) and compare the effects of IE-QE with intraperitoneal quercetin (IP-QE) in an experimental testicular TD rat model.

Kai-Kai Chi and Wen-Hui Zhang contributed equally to this work.

Financial Disclosure: *The authors declare that they have no relevant financial interests.*

Funding Support: *This study was supported by the Science and Technology Research Project of the Education Department of Henan Province (No. 13A360578).*

From the Department of Urology, The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, China; the Department of Hematology, Henan Provincial People's Hospital, Zhengzhou, China; and the Department of Hematology, The Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China

Address correspondence to: Guang-Ce Wang, Department of Urology, The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou 450000, China. E-mail: wgc460@medmail.com.cn

Submitted: June 5, 2016, accepted (with revisions): September 12, 2016

http://dx.doi.org/10.1016/j.urology.2016.09.017
0090-4295

METHODS

Animals

Twenty-four male Wistar albino rats (3.0-3.5 months old) weighing between 250 and 300 g were used in the study. The rats were fed a standard diet and housed under standard laboratory conditions at a temperature of 22 ± 2°C, relative humidity of 60%, and 12-hour light-dark cycle. The rats were acclimatized for 1 week before the experiment.

The experimental protocol was reviewed by the Ethics Committee of The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, and the study was conducted according to the national animal welfare guidelines. The rats were randomly divided into 4 groups of 6 rats each: sham (S), TD, TD with IP-QE treatment, and TD with IE-QE treatment.

Surgical Procedures

Animals were anesthetized with an intramuscular injection of chloral hydrate (100 mg/kg) and xylazine (5 mg/kg), and they breathed spontaneously throughout the surgical procedures. The right testis was exposed through an incision in the scrotum. Torsion of each testis was performed by twisting the testicular cord 720° clockwise. The testis was then fixed to the scrotum, and the scrotal incision was closed. The scrotum was reopened after 90 minutes of testicular torsion and the right testicular cord was restored to its anatomical position with the testis replaced in its normal position. QE (20 mg/kg) (Sigma, St. Louis, MO) was injected intraperitoneally after 60 minutes of torsion in the IP-QE group. QE (20 mg/kg) was injected epididymally after 60 minutes of torsion in the IE-QE group. All rats were eventually sacrificed by exsanguination 24 hours after the surgical procedure, and testicular tissue and blood samples were obtained for further analyses.

Biochemical Parameters

All procedures were performed at 4°C, and ice packs were used to maintain the temperature during homogenization. The testicular tissues were washed with cold saline and cut into small pieces, followed by homogenization in ice-cold 0.2 mmol/L Tris-HCl. The homogenates were centrifuged for 8 minutes at 3000 *g* and the supernatants were used for subsequent analyses.

Glutathione Peroxidase (GPx) Enzyme Activity

GPx enzymatic activity was measured by the addition of H_2O_2 to the reaction mixture containing reduced glutathione, nicotinamide adenine dinucleotide phosphate, and GPx. The changes in absorbance at 340 nm were monitored. The activity of the enzyme was expressed as unit per milligram protein.

Tissue Malondialdehyde (MDA)

MDA levels in the tissue samples were determined according to the method of Uchiyama and Mihara.[12] Tetramethoxypropane was used as a standard and tissue MDA levels were calculated as nanomole per gram of wet tissue.

Total Antioxidant Status (TAS)

TAS was determined using an automated method developed by Erel.[13] Serum TAS levels were calculated as millimole Trolox equivalent per liter.

Total Oxidant Status (TOS)

TOS was also measured using an automated colorimetric method developed by Erel.[14] Serum TOS levels were calculated as micromole H_2O_2 equivalent per liter.

Oxidative Stress Index (OSI)

The OSI was defined as the ratio between the TOS and TAS. According to Aycicek et al,[15] TAS levels (millimole Trolox equivalent per liter) were converted to micromole Trolox equivalent per liter to perform the calculation. The OSI was calculated as follows: OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, μmol Trolox equivalent/L) × 100].

Histopathological Examination

The testicular tissue samples were fixed in Bouin solution and embedded in paraffin. Five micrometer-thick sections were cut, stained with hematoxylin and eosin, and examined with a light microscope (Olympus, Tokyo, Japan). Histologic changes in the testes were scored according to the testicular biopsy score count by Johnsen[16] and the grading system,[17,18] respectively.

Evaluation of Germ Cell Apoptosis

Single-cell suspensions of testicular cells were prepared by mechanical isolation and subsequent filtration. Germ cell apoptosis was determined using the Annexin V-FITC and PI apoptosis detection kit (Beyotime, Jiangsu, China). The proportion of apoptotic cells was measured using a flow cytometer (Becton Dickinson, Franklin Lakes).

Statistical Analysis

The Shapiro-Wilk test was used to assess the normal distribution of data, and the Levene test was used to assess the homogeneity of variance. Analysis of variance with Bonferroni correction was performed for comparisons among groups. All statistical analyses were performed using the SPSS version 17.0 software (SPSS, Chicago). The results were considered statistically significant at $P < .05$.

RESULTS

Biochemical Parameters

The results from the biochemical analyses of all groups are presented in Table 1. Testicular tissue MDA and serum TOS and OSI were higher in the TD group compared with those in the S group ($P < .05$). Testicular tissue GPx and Serum TAS were higher in the S group compared with those in the TD group ($P < .05$). We observed significant testicular damage in the TD group compared with the other groups. Tissues from the QE-treated groups showed significantly less IR injury, with decreased levels of MDA, TOS, and OSI, respectively, compared with those in the TD group ($P < .05$). No