Journal of Pediatric Surgery (2012) 47, 1716–1723

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Comparison of the protective effect of dipyridamole and acetylsalicylic acid on long-term histologic damage in a rat model of testicular ischemia-reperfusion injury ☆

Ersagun Karagüzel[a,*], Ömer Kutlu[a], Esin Yulug[b], Sevdegül Mungan[c], İlke Onur Kazaz[a], Doğan Sabri Tok[a], Güner Kemal Özgür[a]

[a]*Department of Urology, Karadeniz Technical University, School of Medicine, 61080 Trabzon, Turkey*
[b]*Department of Histology and Embryology, Karadeniz Technical University, School of Medicine, 61080 Trabzon, Turkey*
[c]*Department of Pathology, Karadeniz Technical University, School of Medicine, 61080 Trabzon, Turkey*

Received 1 December 2011; revised 5 January 2012; accepted 31 January 2012

Key words:
Dipyridamole;
Acetylsalicylic acid;
Testicular torsion;
Rats;
Reperfusion injury

Abstract

Purpose: Ischemia reperfusion injury arising from testicular torsion results in a loss of spermatogenesis and a significant increase in germ cell apoptosis. We investigated the effects of dipyridamole and acetylsalicylic acid (ASA), 2 well-known platelet inhibitors, on testicular ischemia reperfusion injury.

Methods: Thirty adult male Sprague-Dawley rats were randomly divided into 5 groups (n = 6 for each group): control, sham-operated, torsion/detorsion (T/D), T/D + dipyridamole, and T/D + ASA. Testicular ischemia was achieved by rotating the left testis 720° clockwise for 2 hours. Thirty minutes before torsion, 10 mg/kg dipyridamole was injected transperitoneally in the T/D + dipyridamole group, and 100 mg/kg ASA was injected transperitoneally in the T/D + ASA group. Sixty days after the initial surgical procedure, ipsilateral orchiectomies were performed for histopathologic examination to determine Johnsen's mean testicular biopsy score (MTBS), mean seminiferous tubular diameter (MSTD), and apoptotic index (AI) in all groups.

Results: Unilateral testicular torsion-detorsion led to a significant decrease in Johnsen's MTBS and MSTD values in the ipsilateral testis and a significant increase in AI values of the T/D group. There were no significant differences between the T/D + dipyridamole and control groups in terms of MSTD and MTBS values. Although an amount of improvement exits in T/D + ASA group, there were significant differences between the T/D + ASA and control group MSTD and MTBS values. There was no significant difference between the T/D + dipyridamole and control groups in terms of AI values ($P > .05$), but the differences between the T/D + ASA and control groups were significant despite a slight decline in AI values of the T/D + ASA group.

Conclusions: Our findings show that the use of dipyridamole before testicular reperfusion has a potentially protective effect against long-term injury in testicular ischemia reperfusion injury.

☆ Conflict of interest: There is no conflict of interest to report.

* Corresponding author. Tel.: +90 0532 5029672; fax: +90 0462 3250518.
E-mail address: ersagunkaraguzel@gmail.com (E. Karagüzel).

0022-3468/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jpedsurg.2012.01.078

Testicular torsion is a urologic emergency; it may be seen in newborn males, children, and adolescents. Germ cell loss may arise as a result of testicular torsion [1]. Even if torsion is successfully corrected surgically, loss of spermatogenesis and a significant increase in germ cell apoptosis may still occur as a result of ischemia-reperfusion (I/R) injury and testicular oxidative stress [2]. Many different drugs have been tried to prevent testicular injury resulting from I/R (sildenafil citrate, dehydroepiandrosterone, propofol, morphine, quercetin, trapidil, erdosteine, etc), but none have been widely used [3-9].

Dipyridamole is a platelet inhibitor with known antithrombocytic effects. It inhibits thrombocyte adhesion and aggregation. In addition to its antiaggregant effects, it also has anti-inflammatory and neuroprotective properties. Dipyridamole has been reported to possess antioxidant properties, but its direct effect mechanism on vascular cells is unknown [10]. Experimental studies have shown that dipyridamole reduces I/R injury induced in the liver and myocarditis [11-13]. Acetylsalicylic acid (ASA) has been well researched and is the most widely used and the least toxic of the platelet inhibitors [14,15]. It is also widely used as an analgesic, anti-inflammatory, and antipyretic agent [16]. Experimental studies show that ASA reduces I/R injury induced in the kidneys and in myocarditis [17,18].

The aim in this study was to investigate the potential long-term protective effects of 2 oral antithrombotic drugs, dipyridamole and ASA, in I/R injury resulting from testicular torsion.

1. Material and methods

Thirty adult male Sprague-Dawley rats weighing between 350 and 400 g were used. Animals were housed in individual cages under standard conditions in a temperature and light-controlled room on a 12-hour light/12-hour dark cycle and allowed consumption of sterile food (animal chow) and water ad libitum. All animal experiments were performed following Karadeniz Technical University Animal Care and Ethics Committee approval, in compliance with the principles of laboratory animal care (National Institutes of Health publication no. 85-23; revised 1985). Dipyridamole was obtained from Koçak Farma (İstanbul, Turkey) and dissolved in 10-mL NaCl immediately before intraperitoneal injection. Acetylsalicylic acid was obtained from Sigma Chemical Company (St. Louis, MO, USA) and dissolved in 2-mL $NaHCO_3$ + 2-mL distilled water immediately before intraperitoneal injection.

1.1. Experimental groups

Thirty Sprague-Dawley rats were divided randomly into 5 experimental groups of 6 animals each: control, sham, torsion/detorsion (T/D), T/D treated with dipyridamole, and T/D treated with ASA.

1.2. Surgical procedure

Surgical procedures were performed under general anesthesia, induced by intraperitoneal injection of 50 mg/kg of ketamine hydrochloride (Ketalar, Eczacibasi, Turkey) under sterile conditions.

Group I (control): This group was constituted to determine baseline values of histopathologic parameters.

Group II (sham-operated): This group was constituted to investigate the effect of surgical stress on spermatogenesis. The left testes were extracted through a scrotal midline incision and then replaced with fixation to the scrotum. The wound was closed using 4-0 silk suture.

Group III (T/D): Torsion was created by rotating the left testis 720° clockwise and maintained by fixing the testis as described by Turner et al [19]. After 2 hours of torsion, the testis was counterrotated to the natural position and replaced into the scrotum.

Group IV (T/D treated with dipyridamole): The same surgical procedure was performed as in group III; in addition, 10 mg/kg dipyridamole (Koçak Farma) was injected transperitoneally once only 30 minutes before detorsion [4].

Group V (T/D treated with ASA): The same surgical procedure was performed as in group III; in addition, 100 mg/kg ASA (Sigma Chemical Co) was injected transperitoneally once only 30 minutes before detorsion [4].

1.3. Histopathologic analysis

The study was concluded 60 days after the surgical procedure. All rats were killed by cervical dislocation after the bilateral testes had been harvested. At the end of the surgical procedure, for histologic evaluation, the testis tissues were immediately fixed in Bouin solution for 24 hours, dehydrated in increasing concentrations of ethanol, and embedded in paraffin for histologic evaluation. Tissues were then sectioned at 5 μm using a microtome (Leica RM 2255; Leica Microsystems, Tokyo, Japan). They were then dewaxed and rehydrated through a graded ethanol series using routine protocols and stained with H&E. All testicular histology was assessed blindly. Light microscopy (Olympus BX51 microscope; Olympus, Tokyo, Japan) was used for the evaluations, and photographs were taken using a light microscope with a camera attachment (Olympus DP 71; Olympus, Tokyo, Japan). Testis sections from each study group were evaluated for structural changes. The mean seminiferous tubular diameter (MSTD) in 10 random microscopic fields of each testicular section was measured under microscope at original magnification ×20 with an Analysis 5 Research program (Olympus Soft Imaging Solutions, Münster, Germany). Twenty of the roundest tubules per testicular section were selected and calculated. Johnsen's mean testicular biopsy score (MTBS) was used to evaluate the histopathologic changes in 20 seminiferous tubules of each testicular section [20]. Johnsen's MTBS was calculated by dividing the sum of all scores by the total number of seminiferous tubules examined.