The effects of unilateral testicular ischemia and hemicastration on contralateral testicular IGF-1 level, histology and lipid peroxidation

Cagri Savas1, I. Faruk Ozguner1, Meltem Ozguner2 & Namik Delibas3
1Suleyman Demirel University, Medical School, Department of Pediatric Surgery, Turkey; 2Suleyman Demirel University, Medical School, Department of Histology and Embryology, Turkey; 3Suleyman Demirel University, Medical School, Department of Biochemistry, Turkey

Abstract. Hemicastration is followed by compensatory hypertrophy whereas unilateral testicular torsion is followed by atrophy in contralateral testicle in rats. Insulin-like growth factor (IGF-1) has important roles in testicular paracrine and autocrine functions. In this study it was aimed to compare ischemic parameters and IGF-1 levels in the contralateral testicle in unilateral spermatic cord ligation, testicular torsion, and hemicastration. 32 wistar rats were equally allocated into sham, ligation, torsion, and hemicastration groups. In ligation group, right spermatic cord was ligated with 3/0 silk suture. In the torsion group, right testis was tcrsed for 720 degrees. In hemicastration group, right orchiectomy was done. 48 hours later left orchiectomy was done in all groups. Malondialdehyde (MDA) and IGF-1 levels were determined in the testicle. Average values of the groups were compared with Anova followed by Dunnett T3 multiple comparison tests. MDA levels were significantly reduced in ligation and torsion groups (p < 0.05). This reduction was more prominent in hemicastration group (p < 0.05). Contralateral testicular IGF-1 levels in ligation and torsion groups were not different compared with the sham group. Left testicular IGF-1 level in the hemicastration group was decreased significantly compared with other groups (p < 0.05). Histological changes evaluated. Contralateral Johnsen’s testicular biopsy scores were significantly decreased in all experimental groups but mean tubular diameter was not changed in all groups.

Key words: Testicular torsion, Hemicastration, IGF-1, Free radicals

Introduction

Unilateral testicular torsion could cause damage to an otherwise normal contralateral testis [1]. However, the pathogenesis of the contralateral testicular damage remains poorly understood. Several theories have been postulated, including autoimmunization, subclinical episodes of contralateral testicular torsion, release of acrosomal enzymes, paired neuroendocrine or vaso-motor response during torsion, presence of an underlying defect in spermatogenesis, and presence of an inherent gonadal abnormality [2, 3]. After hemicastration, compensatory hypertrophy of the remaining testis tissue occurs in prepubertal rats but it is not clear whether compensatory growth occurs in adult animal. Recently, electromagnetic and radioisotopic measurements of testicular blood flow have shown that unilateral testicular torsion causes a decrease in contralateral nontorted testicular blood flow, which gradually increases after detorsion [4, 5].

Although follicle-stimulating hormone and luteinizing hormone are the major regulators of testicular insulin-like growth factor 1 (IGF-1; somatomedin C) production, growth hormone (GH) may play an indirect role by potentiating the actions of the gonadotropins in regulating testicular IGF-1 content [6]. Serum levels of IGF-1 increase with age and pubertal development. Mammalian cells and tissues are protected from the deleterious consequences of oxidative stress by complex systems of enzymes and antioxidants [10]. Under normal conditions, free radicals generated during cell metabolism are rapidly scavenged by endogenous antioxidant enzymes [5]. The most important indicators of ischemia-reperfusion injury are the increase in lipid peroxidation and changes in the antioxidant enzyme activities The
aim of this study was to investigate spermatogenesis, morphometry, IGF-1 and malondialdehyde (MDA) levels in the contralateral testis in rats with ipsilateral testicular torsion, hemicastration or spermatic cord ligation.

Materials and method

Animal model

This study was carried out on 32 adult male albino Wistar rats weighing between 240–260 g. The animals were acquired from the university vivarium sources and were housed in individual cages in a temperature and light-dark cycle controlled environment with free access to food and water. All animals received humane care, in compliance with the ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Experimental design

Animals were randomly assigned to experimental groups and were operated on in random order. Investigators blinded to the experimental groups made outcome assessments. Rats were randomly allocated into four groups where each one consisting of eight rats:

1. Sham: sham operation group.
2. Torsion: right testicular torsion group.
3. Ligation: right spermatic cord ligation group.

Surgical procedure

Surgical procedures were performed using a sterile technique under 5% ketamine hydrochloride anesthesia (50 mg/kg, intramuscular). A right-sided abdominocrotal incision was performed to get access the right testis after skin shaving and preparation with 10% povidone-iodine solution. Testicular torsion was created in the torsion group by twisting the right testis 720° in counterclockwise direction and maintained by fixing the testis to the scrotum with a 4/0 atraumatic silk suture passing through the tunica albuginea and dartos. Right spermatic cord was ligated with a 4/0 silk suture including spermatic vessels and ductus deferens in the ligation group. Right orchiectomy was done in the hemicastration group. After 48 hours, the rats were sacrificed with an overdose of pentobarbital sodium and contralateral (left) orchiectomy was performed. Left testes were divided longitudinally into two halves for histopathologic evaluation and biochemical assays. Specimens for histopathologic evaluation were individually immersed in Bouin’s fixative. Specimens for biochemical assays were placed in glass bottles with rubber caps, labeled and stored at the –78°C until assay.

Histopathology

Left testes collected after 48 hours were individually immersed in Bouin’s fixative (7.5 ml of saturated picric acid, 2.65 ml of glacial acetic acid and 2.5 ml of 7% formaldehyde) and kept at 4°C for five days after which they were dehydrated in alcohol and embedded in paraffin. Five µm sections were obtained, deparaffinized and stained with hematoxylin and eosin. Three slides prepared from the upper, lower, and midportions of the testes were evaluated completely for each testes. Mean tabuler diameter (MTD) was measured in micrometers (µm) and Johnsen’s score [1–10] was calculated [7].

Biochemical procedure

Briefly, the frozen sample of rat testis was weighed and homogenized (Ultra Turrax T25, Germany) (1:10, w/v) in 100 mmol/L phosphate buffer (pH 7.4) containing 0.05% sodium azide in an ice bath. The homogenate was sonicated (Bandelin, Germany) for 30 seconds and centrifuged (5000 g for 10 minutes). The supernatant was frozen at –78°C in aliquots until used for biochemical assays. The protein content of the supernatant was determined using the Lowry method [8].

Insulin like growth factor-1 assay

The concentration of insulin like growth factor-1 in the supernatant was determined using immunoradiometric assay kit for IGF-1 (Immunotech, Ref. No: 3516, Marseille, France) according to the manufacturer’s specifications. The immunoradiometric assay of IGF-1 is a ‘sandwich’ type assay. Mouse monoclonal antibodies, directed against two different epitopes if IGF-1 and therefore, not competing, are employed in the kit. Samples, extracted with acid ethanol, and standarts are incubated in tubes coated with the first