The Levels of Oxidative Stress Biomarkers in Rats as a Response to Different Techniques of Testicular Biopsy

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Abstract Some biopsy techniques may cause oxidative stress. Therefore, we aimed to evaluate the possible role of testicular biopsy-induced damage on production of reactive oxygen species using quantitative and biochemical methods. Adult male Wistar albino rats were randomly divided into five groups. Group 1 (sham) ($n=6$) was sham operated. Group 2 ($n=7$) underwent fine-needle aspiration testicular biopsy. Group 3 ($n=6$) underwent microscopic testicular sperm extraction (micro-TESE). Open testicular biopsy was performed to rats in group 4 (macro-TESE) ($n=8$). Group 5 ($n=7$) underwent Tru-cut biopsy. Six weeks after the initial operations, orchietomies were performed. Oxidative stress biomarkers such as superoxide dismutase (SOD) and catalase (CAT) activities, and malondialdehyde (MDA) in biopsy samples were measured as spectrophotometric. Compared with group I, SOD and CAT activities, and MDA levels were elevated significantly in the fine-needle aspiration group, in the macro-TESE group and in the Tru-cut biopsy group

(groups II, IV, and V), ($p<0.05$). However, there were no significant differences between group I and group III ($p>0.05$). These data suggest that micro-TESE biopsy is the best technique among all others. All of the biopsy techniques except micro-TESE may cause the overproduction of reactive oxygen species. We consider that the increased antioxidant enzyme activities (CAT, SOD) may reflect the cellular response against oxidative stress in these groups.

Keywords Testicular biopsy · Reactive oxygen species · Oxidative stress · Micro-TESE

Introduction

Testicular biopsy is an integral part of the treatment of infertility in men such as in men with azoospermia. In the past, open testicular biopsy (macro-testicular sperm extraction (TESE)) has been the standard procedure to obtain testicular tissue for histopathological examination [1]. Nowadays, different surgical techniques such as open testicular sperm extraction (macro-TESE), microscopic TESE (micro-TESE), and fine-needle aspiration biopsy can be used to extract spermatozoa from the testis. Concerns have been raised regarding the potential complication of these biopsy techniques, including damage on testicular tissue.

Oxygen is required to support life, but its metabolites, such as reactive oxygen species [ROS], can modify cell functions and endanger cell survival [2]. For this reason, ROS is inactivated continuously to maintain only the small amount necessary to maintain normal cell function [3]. There is a balance between the different antioxidants and oxidants at normal status [4]. Oxidative stress develops when oxidants outnumber antioxidants, and peroxidation products occur, and these may cause pathologic effects [5, 6]. ROS are believed to have potential toxic effects at high levels on sperm quality and function in the male reproductive tract [7–9]. Oxidative stress

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is a cellular condition associated with an imbalance between the production of free radicals, mainly ROS, and the scavenging capacity of antioxidants. Low levels of ROS are essential for normal fertilization, capacitation, hyperactivation, motility and acrosome reaction of sperm cells [9, 10]. When the production of ROS exceeds the available antioxidant defence, significant oxidative damage occurs to many cellular organelles by damaging lipids, proteins, DNA and carbohydrates. Oxidative stress leads to spermatozoal dysfunction, DNA damage, infertility and sperm cell death [11].

It is also believed that testicular injury such as biopsy causes damage of testicular tissue. To our knowledge, this is the first report of the levels of oxidative stress in rats from which various testicular biopsies were taken previously. In the present study, we aimed to figure out which testicular biopsy technique causes less testicular damage.

Materials and Methods

Animals

Experiments were performed on adult male Wistar albino rats weighing 240–405 g, obtained from breeding stock. All animals were housed two per cage (20×18×13 cm high) with free access to standard pellet food and filtered water, at 23–26 °C. All observations were made between 9:00 and 17:00 hour in a quiet room. Male rats were tested, since the major interest of this study was to investigate the levels of oxidative stress in the serum of rats from which various testicular biopsies were taken. The institutional ethical committee approved this study.

Experimental Procedure

The animals were anesthetized with intraperitoneal ketamine (45 mg/kg). The animals maintained an imposed position with both front limbs extended and resting on a 3-cm-high wood bar [0.9 cm in diameter]. The scrotum was entered through a midline incision under sterile conditions. The tunica vaginalis was opened, and the left testicle was delivered to surgical field. Wistar albino rats were divided into five groups consisting of ten rats in each. Group 1 (sham) ($n=6$) was sham operated: the left testicle was incised until the tunica albuginea and then replaced in the scrotum. Group 2 ($n=7$) underwent fine-needle aspiration testicular biopsy. [12]. Percutaneous aspirations (testicular sperm aspiration) were made with a high-suction glass syringe and a 23-gauge needle. Group 3 ($n=6$) underwent micro-TESE. Open testicular biopsy was performed to group 4 (macro-TESE) ($n=8$). Group 5 ($n=7$) underwent Tru-cut biopsy. The animals were returned to their home cages between determinations. Six weeks after the initial operations, orchiectomies were performed.

Biochemical Analysis

The tissue samples were immediately excised, weighed, perfused with 1.15 % ice-cold KCl, minced, then homogenized in five volumes (w/v) of the same solution, using a Heidolph 50110 R2R0 homogenizer. Antioxidant systems and lipid peroxidation (LPO) assays were performed on the supernatant preparation in a Sorvall RC-2B centrifugation of the homogenate at 14,000 rpm for 30 min at +4 °C [13].

Evaluation of Biochemical Parameters

1. Catalase (CAT) Activity

CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler [14]. The assay medium consisted of 1 M Tris–HCl–5 mM Na₂EDTA buffer solution (pH8.0), 1.0 M phosphate buffer solution (pH7.0) and 10 mM H₂O₂. CAT activity was expressed as unit per milligram protein.

2. Superoxide Dismutase (SOD) Activity

SOD activity was measured according to the method described by Fridovich [15]. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with *p*-iodonitrotetrazolium violet (INT) to form a red formazan dye which was measured at 505 nm. The assay medium consisted of the 0.01 M phosphate buffer; CAPS (3-cyclohexylamino-1-propanesulfonic acid) buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with pH10.2; solution of substrate (0.05 mM xanthine, 0.025 mM INT) and 80 U/L xanthine oxidase. SOD activity was expressed as unit per milligram protein.

3. Measurement of Lipid Peroxidation Level

LPO level in the tissue samples was expressed as malondialdehyde (MDA). It was measured according to procedure of Ohkawa et al. [16]. The reaction mixture contained 0.1 ml of sample, 0.2 ml of 8.1 % sodium dodecyl sulphate, 1.5 ml of 20 % acetic acid and 1.5 ml of 0.8 % aqueous solution of TBA. The mixture pH was adjusted to 3.5, and the volume was finally made up to 4.0 ml with distilled water; 5.0 ml of the mixture of *n*-butanol and pyridine (15:1, v/v) was added. The mixture was shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm.

Protein Measurement

Total protein contents were determined by the method of Lowry [17], using bovine serum albumin (Merck-Darmstadt, Germany) as standard.

Statistical Analysis

Results are expressed as means $\pm$ SD. The significance of the difference in means was determined by Mann–Whitney *U* and one-way ANOVA tests. The level of significance was set at $p < 0.05$.

Results

All biopsies yielded adequate tissue for histopathological evaluation. Post-biopsy infections were not seen, and extra-/intratesticular haematomas were not noted. All animals tolerated the study well. Six weeks after testicular biopsies, the ipsilateral and contralateral testicular sizes and appearances in all groups were unchanged when compared with pre-biopsy observations.

Compared with the sham group (group I), the SOD and CAT activities, and MDA levels were elevated significantly in the fine-needle aspiration group, in the macro-TESE group and in the Tru-cut biopsy group (group II, IV and V) ($p < 0.05$). However, there were no significant differences between the sham group (group I) and micro-TESE group (group III) as seen Table 1 ($p > 0.05$).

Discussion

Azoospermia, the absence of sperm in ejaculated semen, is the most severe form of male-factor infertility and is present in approximately 5 % of all investigated infertile couples. The advent of intra-cytoplasmic sperm injection (ICSI) has transformed the treatment of this type of severe male-factor infertility. Sperm can be retrieved for ICSI from either the epididymis or the testis, depending on the type of azoospermia [18]. Various ways of sperm retrieval include microsurgical testicular

sperm extraction (micro-TESE), open testicular biopsy (macro-TESE), testicular fine-needle aspiration biopsy and Tru-cut biopsy [18]. Complications associated with surgical sperm retrieval techniques are haematoma and fibrosis, identified at ultrasound in the months immediately following the procedure [19]. Testicular atrophy and devascularization are considered to be long-term physiological complications [20].

ROS are endogenous oxygen-containing molecules formed as normal products during aerobic metabolism [21]. Free radical production occurs continuously in the cell during metabolism. These radicals are in part toxic to the cell and cell membranes; however, they are normally controlled by countervailing biologic mechanisms. Severe oxidative stress produces ROS and induces uncontrolled lipid peroxidation. Increased formation of ROS and/or decreased antioxidant defence can be defined as oxidative stress, which is widely recognised as an important feature of many diseases such as diabetes mellitus, cancer and renal failure [22]. SOD and CAT are endogenous antioxidants, which protect cells from oxidative stress [23]. Scavengers of ROS and antioxidants are important in treating and preventing the damage caused by such stress [24]. LPO is one of the most important expressions of oxidative stress induced by ROS. MDA is an indicator of lipid peroxidation. The relationship between the production of ROS and infertility in males has been known. Semen analysis shows that fertile males have higher antioxidant capacity than infertile controls. This event indicates that impaired antioxidant capacity may play a key role in fertility disorders [25, 26]. ROS are postulated to be mediators of damage to spermatozoa and are related to decreased motility, abnormal morphology and reduced capacity for sperm–oocyte penetration [26]. A balance normally exists between ROS production and antioxidant scavenging activities in the male reproductive tract. As a result of such balance, only minimal amounts of ROS remain, and they are needed for the regulation of normal sperm functions, such as sperm capacitation, the acrosome reaction and sperm–oocyte fusion [27].

ROS play an important physiological role in the activities of proteins which are vital for the differentiation, function and proliferation of sperm. In semen of fertile men, the amount of ROS is controlled by seminal antioxidants. The main sources of overproduction of ROS in the semen are leucocytes and morphologically abnormal spermatozoa [28]. Some authors demonstrated that high levels of ROS were detected in the semen of 25 to 40 % of infertile men [29]. In this investigation, the oxidative stress response of the adult rat testis in vitro was evaluated. The possible role of testicular biopsy-induced damage on production of ROS was exposed using quantitative, histopathological approaches. We wanted to show oxidant/antioxidant levels on sera of rats from which various testicular biopsies were taken. Here, we tested whether or not there is a

Table 1 The levels of oxidative stress biomarkers in all groups

Groups	Oxidative stress biomarkers		
	CAT	SOD	MDA
Group 1 (sham)	7.50 $\pm$ 2.24	7.23 $\pm$ 1.39	0.22 $\pm$ 0.04
Group 2 (fine-needle aspiration)	18.56 $\pm$ 3.88	10.03 $\pm$ 2.79	0.35 $\pm$ 0.04
Group 3 (micro-TESE)	8.75 $\pm$ 1.56*	8.52 $\pm$ 1.47*	0.22 $\pm$ 0.02*
Group 4 (macro-TESE)	18.93 $\pm$ 2.88	10.88 $\pm$ 2.43	0.57 $\pm$ 0.17
Group 5 (Tru-cut biopsy)	17.83 $\pm$ 6.93	10.45 $\pm$ 2.27	0.41 $\pm$ 0.07

Values were expressed as mean $\pm$ SD. The results for CAT and SOD were expressed as unit per milligram protein, and the results for MDA were expressed as nanomole per milligram protein

* $p > 0.05$ indicating no significant difference in MDA, CAT and SOD levels of group 3 compared to the sham group

detrimental effect of testicular biopsies in terms of oxidative stress. The micro-TESE biopsy technique was founded to be the least damage-causing technique among all biopsy techniques (Table 1). The other techniques used were less likely to perform well. Because, in our study, MDA levels of the micro-TESE group were similar to the sham group, there was no significant oxidative stress in the micro-TESE group. However, increased CAT, SOD and MDA levels in fine-needle aspiration, macro-TESE and Tru-cut biopsy groups were found higher than in the sham group. Techniques in these groups [2, 4, 5] may cause oxidative stress. Besides, increased antioxidant enzyme activities (CAT, SOD) may demonstrate the cellular response against oxidative stress in these groups.

In conclusion, all techniques except micro-TESE may cause the overproduction of reactive oxygen species. Thus, we can say that micro-TESE decreases the adverse oxidative effects on testicular tissue. Micro-TESE appears to be an effective method for preserving testicular tissue and the best technique that causes less oxidative stress.

Conflict of Interest We certify that there is no actual or potential conflict of interest in relation to this article.

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