RESEARCH ARTICLE

Effect of low dose ionizing radiation-induced oxidative stress on sperm count and sperm motility in male mice

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Abstract:

Male infertility is contributed to approximately 50% of infertility worldwide. Reactive oxygen species (ROS) is known as one of the major factors contributing to male infertility. This animal modeling study aimed to evaluate the effects of oxidative stress induced by low dose ionizing radiation exposure on sperm count and sperm motility. Twelve adult male ICR mice were randomly divided into two groups (control group, n=6 and irradiated group, n=6). Both groups received filtered tap water and a normal pellet diet for five days of the acclimatization period prior to total body exposure of 200 µGy x-ray on the 6th day. All the mice were sacrificed in cervical dislocation. The epididymis was evaluated for sperm analysis using a computer-assisted sperm analyzer (CASA) system. The sperm count and sperm motility decreased significantly in the irradiated group as compared to the control group (95.8 ± 40.2 million vs 49.3 ± 19.3 million, $p < 0.05$ and 40.8 ± 13.3 % vs 23.2 ± 10.3 %, $p < 0.05$), respectively. Low dose ionizing radiation-induced oxidative stress would reduce the sperm count and sperm motility as a consequence of the increased level of ROS in the testis.

Keywords: Low dose ionizing radiation; oxidative stress; male infertility; sperm count; sperm motility

1. INTRODUCTION

Ionizing radiation has become a necessary part in modern medicine for medical diagnostic and therapeutic. Ionizing radiation breaks the chemical bonds of biological macromolecules following its interaction with living cells. Ionizing radiation can affect proteins, nucleic acids and complex lipids as a result of the generation of reactive oxygen species (ROS) via radiolysis of water or alteration of mitochondrial functions [1]. ROS is highly chemical entities performing vital signaling functions and occurs in nature in the cells [2, 3]. ROS activity is regulated by an amount of enzymatic and non-enzymatic antioxidants. The imbalance of increased generation of ROS and antioxidant mechanisms leads to oxidative stress as manifested by increased oxidative molecular damage to biomolecules [4]. As a consequence, the oxidative damage leads to reduce growth, fertility and survival [5]. Low dose ionizing radiation is defined as ionizing radiation with dose < 100 mGy [6]. The key sources of low-dose radiation exposure to the general population are diagnostic medical exposures and natural background radiation [7]. Low dose ionizing radiation has been significantly associated with the generation of ROS [2, 8]. Previous reports have shown significant correlation between genetic damage and oxidative damage following the effect of ROS generated from ionizing radiation [5, 9].

Male infertility as a result of ROS generation occurs via two mechanisms. Initially, ROS damages the sperm membrane and decreases sperm mobility thus affecting their fusion capability with the oocyte. Furthermore, transmission of sperm information to the embryo is interrupted as a result of DNA damage of the sperm [10]. Increased levels of ROS have been reported in the semen of approximately 25 - 40% of infertile men [11].

Many studies have addressed the effect of several inducers of oxidative stress on the sperm quality in male mice such as radiofrequency electromagnetic radiation [12], radioactive cesium [13] and gamma rays [14, 15]. Nevertheless, limited studies have futher investigated such effect from acute low dose x-ray irradiation. Hence, the present study was designed to investigate in vivo effects of low dose ionizing radiation induced oxidative stress on sperm count and sperm motility in mice.

2. MATERIALS AND METHODS

2.1 Animal handling

The animal study was conducted in accordance with the criteria set by Universiti Teknologi MARA Committee of Animal Research and Ethics (UiTM CARE) concerning the use of experimental animals. Twelve (n=12) healthy six weeks old male Mus musculus mice species weight of 28 - 30 grams were obtained from Laboratory Animal Facility and

Management (LAFAM), UiTM Selangor Branch, Puncak Alam Campus. Mice were individually housed under standard conditions (controlled atmosphere with a 12 hrs light/dark cycle at 20 – 25 ºC). The mice were acclimatized for five days and normal pellet diet along with filtered tap water was given Ad libitium.

2.2 Animal irradiation

The mice were divided randomly into irradiated and control group. The irradiated group (n=6) were exposed with single fractionated of 200 µGy x-ray for total body irradiation on the day sixth. The low dose ionizing radiation was irradiated by using x-ray (Philips Bucky Diagnost) performed under the guidelines provided by the Centre of Medical Imaging, Faculty of Health Sciences, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus. The irradiated and control mice were sacrificed by cervical dislocation and their epididymis tissues were immediately removed for further analysis following the treatment procedure.

2.3 Semen collection and sperm analysis

For preparation of epididymal sperm, the mice were sacrificed by cervical dislocation. About 3 mm cauda epididymis was dissected and placed in 1 ml of medium M16 [14]. The semen samples were incubated at 37ºC for 15 min. Subsequently, the sperm count and motility were analyzed by a Hamilton-Thorne computer assisted sperm analyzer (CASA) (Hamilton Thorne Research, Beverly, MA, USA) (Figure 1).

Figure 1: Sperm analysis using Hamilton-Thorne computer assisted sperm analyzer (CASA)

2.4 Statistical analysis

All data were analyzed using SPSS software statistical package version 21 (SPSS Inc. Chicago, IL, USA). The data were tested for normality and analyzed with the Student's Independent T-test. The results were expressed as the mean ± standard deviation (SD) with a p -value < 0.05 was deemed statistically significant.

3. RESULTS AND DISCUSSION

Significant reduction of sperm count and sperm motility were observed in the irradiated group as compared to the control group ($p < 0.05$) as shown in Table 1. Upon irradiation, sperm count and sperm motility were reduced by 48.5% and 43.1%, respectively.

Table 1: Mean sperm count and sperm motility of the mice in control and irradiated groups following low dose ionizing radiation

Sperm analysis	Control group	Irradiated group	Mean Difference (95% CI)	p-value
Sperm count (10^6)	95.8 ± 40.2	49.3 ± 19.3	46.5 (5.81 - 87.19)	0.029*
Sperm motility (%)	40.8 ± 13.3	23.2 ± 10.3	17.7 (3.36 - 32.97)	0.028*

The results are expressed as mean±standard deviation.
*Denotes significant result with $p < 0.05$

In the present study, we investigated the effects of acute low dose ionizing radiation (200 µGy) on the sperm quality to understand the underlying toxicity of low dose irradiation as a result of oxidative stress. Testicular tissue becomes vulnerable to oxidative stress-induced pathologies due to the inherent abundance of highly unsaturated fatty acids, high metabolic activity, high mitotic activity and the presence of potential ROS- generating systems [17, 18]. Testicular tissue is one of the most radiosensitive tissues, which can be significantly functionally impaired by low doses of radiation as little as 0.1 Gy [19]. Evidence suggest that ionizing radiation affects the testicular tissue by disturbing the normal spermatogenic metabolism, proliferation and differentiation, which results in mutagenesis or apoptosis of radiosensitive cells, low sperm counts and defective sperm function [20].

Oxidative stress is known as a contributing factor in the development of male infertility and defecting sperm function [21]. Various factors inducing oxidative stress in animal models including infections, tetracycline, streptozotocin (STZ), chromic acid, bisphenol and tertiary-butyl hdyroperoxide (TBHP) have been reported [22–27]. As x-ray exposure leads to ROS formation, hence x-ray irradiation is a reliable method for induction of ROS in animal and human studies.

Epididymal sperm counts and motility are a widely used, simple and sensitive method of assessing the effects of male reproductive toxicants [28] and these parameters are useful in diagnosing male infertility [12]. The present study is in accordance with the previous studies investigating the effect of x-ray irradiation on human sperm quality. Ionizing radiation has been shown to reduce the total number of spermatozoa and the number of motile spermatozoa [14]. Exposure to ionizing radiation leads to abnormalities in spermatogenesis including low sperm count, produce abnormal spermatozoa, and damaged sperm function [29]. Study by Kumar et al. [30] found that low dose ionizing radiation from occupational exposure changes the motility pattern of sperm without affecting the fertility. On the

contrary, higher dose (> 2Gy) of ionizing radiation was denoted from those studies as compared to the present study. A mice model study by Mohamed et al. [31] reported that acute low dose ionizing radiation (100 μGy) would induce oxidative stress. Hence our findings postulate that acute low dose ionizing radiation could significantly affect the epididymal sperm counts and sperm motility.

4. CONCLUSION

Low dose ionizing radiation-induced oxidative stress would lead to decrease the sperm count and sperm motility as a result of increased level of ROS in the mice testis. This finding may serve as important implication for estimating risks of male infertility related to low dose ionizing radiation among radiation workers. This study model may be proposed as a valuable tool for further investigation on the effects of various antioxidants activities on germinal tissues. The sperm analysis on sperm count and sperm motility without histological analysis was insufficient to evaluate the effect of ionizing radiation-induced oxidative stress on the sperm quality. Hence, further investigation on other kinematic parameters of spermatozoa including progressive motility, linearity and curvilinear velocity integrated with histological analysis of sperm would be valuable in the future investigation of the effect of low dose ionizing radiation-induced oxidative stress on the sperm quality.

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