Investigating Effect of Vitamins E and C on Sexual Cell Lineages in Adult Male Rats under Oxidative Stress due to Sulfasalazine

Keshavarz Mojtaba, Kargar Jahromi Hossein, Khodaparast Zahra, Bathae Seyed Hamid, Saberi Roza, Azhdari Sara, Farzam Mohammad

1,2Jahrom University of Medical Sciences, Jahrom, Iran.
1Young Researchers Club Elite, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
1Young Researchers Club Elite, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
1Developmental Biology, Islamic Azad University, Arsanjan, Fars, Iran
1Institution of Supreme Education and Industry of Maragheh, Iran.
1Department of Biology, Islamic Azad University, Jahrom, Fars, Iran.
1Department of Anatomy and Embryology, International Branch, Shiraz University, Shiraz, Iran.

ARTICLE INFO

ABSTRACT

Background & Objective: Sulfasalazine is a drug used for treatment of inflammatory diseases such as inflammatory bowel disease and is prescribed long term for patients. Consumption of this drug causes a range of side effects such as creating sterility in men. Therefore, in this study the effect of vitamins E and C in the prevention of the effects of sulfasalazine on spermatogenesis of male rats was studied. Materials & methods: Adult male rats (150-200 gr), Wistar race, were divided into 5 groups of 8 (control group and four experimental groups). Rats in experimental groups 1, 2, 3 and 4 received sulfasalazine (600 mg/kg) daily for 14 consecutive days via gavage. Also, during this period rats of experimental group 2 in addition to sulfasalazine received vitamin C (20 mg/kg) daily, experimental group 3 received vitamin E (200 mg/kg) daily, and experimental group 4 received vitamin C (20 mg/kg) plus vitamin E (200 mg/kg daily) via gavage. Rats of control group just received regular food and water. At the end of the period, counting sexual cells was performed by light microscopy on testis sections. At the end of experiment, the results were analyzed statistically by ANOVA test and Duncan's test, P≤0.05 was considered significant. Results: Results show that sulfasalazine consumption has an effect on number of sex cells and created significant reduction in the P≤0.05 level, and the combination of vitamins E and C prevents this reduction. Discussion: Results of this study suggest that sulfasalazine with increased oxidative stress causes impaired spermatogenesis, and vitamins E and C by decreasing oxidative stress prevents the damaging effects of sulfasalazine on spermatogenesis.

INTRODUCTION

Investigating the causes of infertility in men and how to prevent the creation of infertility in a community is a valuable aid to patient who is suffering infertility. Sulfasalazine is a drug used for the treatment of inflammatory bowel disease and is also used for treating diseases such as ulcerative colitis, Crohn’s disease and also rheumatoid arthritis. These diseases are chronic and relapsing and patients should use this medication their entire life which will lead to dangerous side effects, one of which is sterility in men. Construction of sulfasalazine is composed of two parts, sulfa pyridine and 5-aminosalicylate which are connected by a bond. Active part of the medicine is 5-aminosalicylate and side effects of medicine are related to sulfa pyridine [1]. Several studies have shown that taking sulfasalazine may cause sterility in men. For the first time Liu and colleagues showed this effect in a patient with ulcerative colitis [2]. In another study Tat and colleagues showed abnormal sperm in the semen of patients treated with sulfasalazine [3]. Fukushima and colleagues examined the mechanism of sterility induced by sulfasalazine [4]. They showed that sperm motility and acrosome reaction are important for fertilization and will be reduced by sulfasalazine. This indicates sulfasalazine's effect is in spermatid and epididymal stage. In a recent study it has been shown that sulfasalazine decreases the number of spermatids and percentage of motile cells in testicular. Moreover, it has been shown that abnormalities exist in...
sperm morphology, like sperm without tails at dose of 600 mg per day. The mechanism of sulfasalazin's effect on infertility has not been identified. Several mechanisms have been proposed regarding the effect of sulfasalazine on spermatogenesis. One mechanism that has been proposed is induced oxidative stress; medications that cause the production of reactive oxygen species may play an important role in reducing the quality and quantity of the semen [4]. Active oxygen causes lipid peroxidation in fatty acids of the cell membrane and so it increases cell membrane permeability which causes immobility and sperm death [5,6]. In a recent study, it has been shown that sulfasalazine decreases the body’s antioxidant defense enzymes such as superoxide dismutase and glutathione reductase [4]. Due to chronic use of sulfasalazine and its effect on sterility, the use of materials that can help prevent side effects of the drug could be one way to solve this problem. Due to increased oxidative stress by sulfasalazine, it appears that antioxidant compounds can be effective in relieving the side effects of the drug. Vitamin E or alpha-tocopherol are antioxidant compounds [7], vitamin E is a fat-soluble vitamin that contains detergent free radicals. One tocopherol molecule breaking antioxidant molecule chains can inhibit two lipid proxisls radical, and so inhibit two chains reaction of proxidation [8]. Vitamin C is an antioxidant compound which is soluble in water and it has a role in neutralization of free radicals and removing oxidative stress. In addition to cleaning free radicals, it causes other antioxidants such as vitamin E and to enter the cycle [9]. Regarding the subject of this study, the goal of this research is to investigate the protective effect of vitamin E and C on the effects of sulfasalazine on spermatogenesis in laboratory rats.

MATERIALS AND METHODS

In the present experimental study, 40 adult male Wistar rats weighting 150 to 200 gr were used. Animals were maintained in Jahrom Animal house in the Jahrom Medical Sciences University in standard conditions (12 hours light/12 hours dark and at temperature 2 ± 22°C). The animals were randomly divided into 5 groups as follows:

The Control group (C): did not receive any medicine and maintenance and nutritional conditions were similar to other groups.

The First experimental group (E1): received sulfasalazine at the dose of 600 mg per kg of body weight by gavage for 14 days. Dose selection was based on drug consumption dose and also previous studies on the effects of induced sterility oxidative stress.

The Second experimental group (E2): In addition to sulfasalazine at a dose of 600 mg per kg of body weight per day received vitamin C at a dose of 20 mg per kg of body weight by gavage 15 minutes prior to receiving sulfasalazine.

The Third experimental group (E3): Once daily received sulfasalazine at a dose of 600 mg per kg of body weight, plus vitamin E at a dose of 200 mg per kg of body weight by gavage 15 minutes prior to receiving sulfasalazine.

The Fourth experimental group (E4): Received sulfasalazine at a dose of 600 mg per kg of body weight per day plus vitamin C at a dose of 20 mg per kg of body weight and 200 mg of vitamin E in the form of gavage. This group was given vitamins E and C 15 minutes before receiving sulfasalazine. It should be noted that during the study, all animals were cared for according to the ethical principles of the animal protection law (SPCA) passed in 2006 in USA (10, 2). Fifteenth days after the start of the study, in sterile conditions, the left and right testes were removed by creating a gap in the lower abdomen, and the left testis was placed in Bouin’s fixative solution for histological examination.

Review of histological testis:

The left testis was removed from Bouin’s fixative solution and tissue molding with paraffin and by microtome cut sections with a thickness of 5 microns were produced and stained with hematoxylin-eosin method. Stained slides were assessed by light microscope by a person who had no knowledge of the category and with the following characteristics: Number of spermatagonia cells, primary spermatocytes, sertoli, leydig, spermatids, and spermatozoa. In each sample 12 semen tubes were examined by light microscope with magnification of ×100. Obtained values were expressed as mean number of cells in each tube.

Statistical analysis:

For data analysis SPSS software version 16 was used, and to compare groups one way ANOVA and Duncan’s test were used. Based on Duncan’s test, if there is a common target in each group there is no significant difference. The P ≤ 0.05 was considered as statistically significant. The means and standard deviations were calculated.
**Results:**

**Histological examination of the testis:**

According to Table 1 it can be seen that sulfasalazine caused a significant reduction in the number of spermatogonia, primary spermatocytes but Vitamin E and C combined together have significantly increased in all experimental groups compared to the control group (Figures 1 to 5).

**Table 1:** The values (mean ± SEM, n=8 each) of testes cells of control rats, and rats treated with sulfasalazine, sulfasalazine +vitamin C, sulfasalazine +vitamin E, or sulfasalazine +vitamin C and vitamin E.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spermatogonia</th>
<th>Primary spermatocytes</th>
<th>Sertoli</th>
<th>Spermatozids</th>
<th>Spermatozoa</th>
<th>Leydig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81.4 ± 1.3a</td>
<td>160 ± 6.1a</td>
<td>36.6 ± 2.5a</td>
<td>118.4 ± 1.7a</td>
<td>79.2 ± 2.1a</td>
<td>33.4 ± 1.2a</td>
</tr>
<tr>
<td>Sulfa</td>
<td>63 ± 1.1b</td>
<td>69.6 ± 2.8b</td>
<td>26.5 ± 0.95b</td>
<td>100.6 ± 2.1b</td>
<td>64.4 ± 1.5b</td>
<td>22.3 ± 0.70b</td>
</tr>
<tr>
<td>Sulfa+vit C</td>
<td>80.9 ± 105a</td>
<td>170.7 ± 2.1c</td>
<td>29.7 ± 1.2c</td>
<td>130.3 ± 3.5c</td>
<td>100.5 ± 1.7c</td>
<td>34.5 ± 0.88c</td>
</tr>
<tr>
<td>Sulfa + vit E</td>
<td>81.6 ± 1.6d</td>
<td>178.5 ± 2.1d</td>
<td>35.3 ± 0.67d</td>
<td>161.5 ± 3.5d</td>
<td>116 ± 1.1d</td>
<td>34.3 ± 0.73d</td>
</tr>
<tr>
<td>Sulfa + vit E &amp; vit C</td>
<td>90.8 ± 1.2e</td>
<td>226.7 ± 7.5e</td>
<td>40.7 ± 0.99e</td>
<td>259.6 ± 3.8e</td>
<td>170.1 ± 2.1e</td>
<td>38 ± 0.86e</td>
</tr>
</tbody>
</table>

Sulfa: sulfasalazine; vit C: vitamin C; vit E: vitamin E. If in each group, there is at least one common letter (a,b,c,d,e), there is no significant difference in this case. Data is given based on the average standard error of the mean. Significant difference is \( P \leq 0.05 \)

**Fig. 1:** Photo micrograph of the seminiferous tubular in control group, staining with Hemotoxylin eosin, magnification of \( \times 100 \), wave mode and the reproductive corrugated epithelium of the tubes 4 to 8 cell layer.

**Fig. 2:** Photo micrograph seminiferous tubular in the sulfasalazine group (experiment 1), Hematoxylin and eosin staining, magnification \( \times 100 \), spermatogenesis is stopped at the stage of primary spermatocytes, thickening of the interstitial space is visible due to fluid retention (edema).
Fig. 3: Photo micrograph seminiferous tubular in sulfasalazine + vitamin C (experiment 2), stained with Hematoxylin and eosin, magnification of ×100, most of the tubes are rich in reproductive epithelium and sperm cells.

Fig. 4: Photo micrograph of seminiferous tubular in sulfasalazine group + vitamin E (experiment 3), Hematoxylin and eosin staining, magnification of ×100, most tubes contain multiple layers of reproductive epithelium, maturity until the final stage of sperm production continues so that all tubes containing the slides of the lumen are filled by these cells.

Fig. 5: Photo micrograph seminiferous tubular in sulfasalazine group + vitamin C + vitamin E (experimental 4), Hematoxylin and eosin staining, magnification ×100, about of 7 tubes seminiferous, perfectly normal epithelium in about 6 to 8 layers.

Discussion and conclusion:
According to the studies, sulfasalazine consumption causes increased oxidative stress and so increases MDA enzyme (which is the final product of lipid peroxidation by reactive oxygen species (ROS)). Subsequently, Ros generate causes stop cell cycle and increasing apoptosis process, thus it will reducing the overall number of sperm [2,11,12]. According to this research, sulfasalazine can cause free radical production (ROS) in the cell. It can be argued that production of free radicals in testicular germ cells which are quite sensitive causes the loss of them. Other studies found that Ros was produced from two different sources in a sperm liquid called damaged spermatozoa cells and activated leukocytes that in high amounts, with disorder in the structure of DNA, reduce the percentage of live sperm and non-sperm binding to the ovum, causing infertility in men [13]. According to this research and also publications by the center of “control and disease protection” it is stated that chemical materials disrupt endocrine system (EDC) with the production of free radicals (ROS) and they are able to reduce oxidative damage to biology molecules such as DNA and protein [14]. It is possible that sulfasalazine by creating free radical (Ros) and mutant in testis tissue, especially the sensitive cells of spermatogonia, primary spermatocyte, spermatid and spermatozoa, cause serious damage and loss of these cells. So there is a risk of damage and loss of interstitial cells and Sertoli in this way. Also, the function of interstitial cells is affected by
sertoli cells [15]. Vitamin E or alpha-tocopherol due to lipid solubility can prevent destructive effects of Ros on sperm parameters [15]. Because a-tocopherol molecule as a chain breaking antioxidant can inhibit two lipid proxils radical and so inhibit chain potential reaction of high oxidation. VitaminC or ascorbic acid is an antioxidant soluble in water that also eliminate free radicals Ros cause the entrance of other antioxidant such as vitamin E to the cycle, thus it is possible that both of these antioxidants can prevent the side effects of sulfasalazine [16].

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the staff of the Research department of Medical Science University of Jahrom.

REFERENCES