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The effect of different doeses of aspirin application on oxidative stress in ovarian tissue

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ABSTRACT

Objective: Aspirin is a non-steroidal anti-inflammatory drug with antioxidative properties. It is recommended to use different doses and durations according to the characteristics of the patient and the type of disease. Therefore, in this study, we aimed to investigate the effect of using aspirin at different doses and for different durations on oxidative stress in ovarian tissue.

Material and Methods: Female Wistar albino rats were divided into five groups. Group 1: control group, no special treatment was applied to the rats in this group. Group 2: 1 mg/kg aspirin was administered orally to the rats in this group every day for 28 days. Group 3: 3 mg/kg aspirin was administered orally to rats in this group every three days. Ggroup 4: 5 mg/kg aspirin was administered orally to rats in this group every five days. Group 5: 7 mg/kg aspirin was administered orally to the rats in this group once a week. After fasting overnight following the last application, the rats were sacrificed, and their ovarian tissues were collected. Malondialdehyde, catalase, total thiol group, and AOPP levels were studied from ovarian tissue.

Results: Group4 and group5 ovarian tissue MDA levels were found to be significantly higher than the other groups ($p<0.05$). There was no significant difference between group1, group2 and group3 ovarian tissue MDA levels ($p>0.05$). Group1 (control group) ovarian tissue AOPP level was found to be significantly lower than all aspirin-administered groups ($p<0.05$). Group2 ovarian tissue AOPP level was found to be significantly lower than group3, group4 and group5 ($p<0.05$). TSG level was found to be significantly higher in group 5 when compared to other groups ($p0<0.05$). Group4 ovarian tissue TSG level was found to be significantly higher when compared to group1, group2 and group3 ($p<0.05$). Group3 and group4 ovarian tissue CAT activity was found to be significantly higher than group1, group2 and group5 ($p<0.05$). When group1, group2 and group5 ovarian tissue CAT activities were compared, no significant difference was found ($p>0.05$).

Conclusion: The application of aspirin at certain intervals rather than daily application may have more positive effects on the antioxidant system. especially taking aspirin at intervals of 3 or 5 days may be more effective

Key words: Aspirin, ovarian tissue, Oxidative stress, Antioxidant, Acetylsalicylic acid

Research Article

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INTRODUCTION

Aspirin (acetylsalicylic acid) is a non-steroidal anti-inflammatory drug, which is widely used for relieving inflammation, fever, and pain (1). Aspirin is broadly used in the treatment of inflammatory diseases such as rheumatoid arthritis and the prevention of cardiovascular thrombosis (2). Aspirin consistently inhibits the production of prostaglandin, which leads to unexpected constriction of arterioles, resulting in tubule ischemia and cell death (3).

Aspirin administration has proven to be beneficial in the chemoprevention of various types of cancer. Unfortunately, aspirin use is still associated with serious consequences and adverse effects. Most importantly, the use of aspirin can specifically induce gastrointestinal toxicity manifested by peptide ulceration, bleeding, and dyspepsia (4).

Aspirin is prescribed for patients undergoing in vitro fertilization (IVF) or intracytoplasmic spray injection. The primary objective of acetylsalicylic acid administration to IVF candidates is to modulate excessive inflammation responses by inhibiting the production of prostaglandins synthesized by Cyxlooxiganesa-2 (5). This medication might increase blood supply to the reproductive organs during the onset of coagulation disorders, resulting in an improved rate of endometrial growth and vascularization (5). Besides, ASA is recommended in patients at high risk of developing preeclampsia as well (5).

Nowadays, aspirin is at the forefront of medical research due to its numerous adverse effects. Increasingly, once-daily medications are emerging. As members of medication therapy management in clinical practice, physicians, pharmacists, and nurses always face a common problem, that is to say, the optimal time and dose for patients to take these drugs. This critical problem reveals the dose, time, and duration of administration for optimizing medications and minimizing their adverse effects.

It has been suggested that low-dose aspirin (80 mg/day) is effective in reducing the risk of cerebral vascular attacks and myocardial infarction, and what's more, it reduces mortality by 25% in patients with cardiac risk factors; however, it has been recommended to use different doses of aspirin for different patients (6).

The treatment timing should be adapted to optimize the therapeutic outcome of the administration of medication as well as to minimize the adverse effects of treatment. Hence, it has been suggested that aspirin therapy administered at different doses and at different times may be beneficial. More frequent administration of aspirin could be an alternative to minimize adverse events (7).

Nonsteroidal anti-inflammatory medications such as aspirin are inhibitors of the cyclooxygenase enzyme and are used in the management of short-term pain and even in the treatment of chronic inflammation. COX enzyme and prostaglandins play a key role in the regulation of female reproductive function. Aspirin use can cause toxicity in many organs such as the liver and kidney. In this study, we aimed to assess the impact of aspirin administration at different doses and durations on oxidative stress in ovarian tissue.

MATERIAL and METHODS

Animals

Forty adult female albino rats (weights ranged 150-200 g, 8-10 weeks) were obtained from the Experimental Animals Unit of Van Yuzuncu Yıl University. Rats were acclimated to laboratory conditions one week before the onset of the experiment. A temperature of 25 °C and 12 hours of light/dark cycles were achieved. Animals had free access to standard pellet food and water. All animal experiments were performed following National Institutes of Health guidelines on the care and use of laboratory animals. The experiment protocol was carried out after obtaining approval from Van Yuzuncu Yil University Experimental Animals Local Ethics Committee (Date :29/04/2021 and Decision number: 2021/04-02).

Experimental Design

Rats were randomly divided into five groups, with 8 in each group.

Group 1; control group, no procedure was applied to the rats in this group.

Group 2; 1 mg/kg aspirin (Bayer AG, Leverkusen, Germany) was administered orally to the rats in this group every day for 28 days.

Group 3; 3 mg/kg aspirin was administered orally to rats in these group every three days.

Group 4; 5 mg/kg aspirin was administered orally to rats in these group every 5 days.

Group 5, 7 mg/kg aspirin were administered orally to rats in this group, for once a week.

At the end of the 28-day study, following 75 mg/kg ketamine (IP) and 5 mg/kg xylazine (IP) administration, the rats were placed on the table in the dorsoventral position and ovarian tissue was dissected for biochemical analysis.

Biochemical analysis

Ovarian tissue was homogenized with 50 mM potassium buffer (pH 7.4). The homogenate was centrifuged at 14000 RPM for 15 minutes at 4°C. The supernatant was used to identify oxidative stress and antioxidant parameters.

Total sulfhydryl content (protein and non-protein Thiols) was measured based on the method of Sedlak and Lindsay (8). In this mehod, TSG were determined with the use of 5,5’ -dithiosis (2-nitrobenzoic acid) (DTNB). DTNB, after reduction by the sulfhydryl group-containing compounds, form a yellow-colored anionic 5-thio-2-nitrobenzoic acid. The absorbance was meusered with spectrophotometer at a wavelength of 412 nm.

AOPP was determined via the method described by Witko-Sarsat (9). AOPP measurement at 340 nm by addition of acetic acid using chloramine-T in the presence of potassium iodide as standart. Ovarian tissue MDA level was measured by the method identified by Ohkava et al. (10) and MDA level was presented as mmol/gr tissue.

The principle of MDA calculation is based on pink color production as a result of the reaction between MDA and thiobarbituric acid (TBA), which is measured by a spectrophometer at 532 nm absorbance. CAT activity was spectrophotometrically analyzed at 240 nm according to the Lartillot method (11).

Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analyzes of the groups were analyzed statistically using the One-way ANOVA followed by post hoc multiple comparisons (Tukey's test) for comparative analysis between the groups.

$P < 0{\cdot}05$ was regarded as statistically significant

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RESULTS

Ovarian tissue Catalase activity and MDA, TSG and AOPP levels are presented in Table1.

Group4 and group5 ovarian tissue MDA levels were found to be significantly higher than the other groups ($p<0.05$). There was no significant difference between group1, group2 and group3 ovarian tissue MDA levels ($p>0.05$). Group 4 and group 5 ovarian tissue MDA levels were similar ($p>0.05$).

Group1 (control group) ovarian tissue AOPP level was found to be significantly lower than all aspirin-administered groups ($p<0.05$). Group2 ovarian tissue AOPP level was found to be significantly lower than group3, group4 and group5 ($p<0.05$).

TSG level was found to be significantly higher in group 5 when compared to other groups ($p0<0.05$). Group4 ovarian tissue TSG level was found to be significantly higher when compared to group1, group2 and group3 ($p<0.05$). Ovarian tissue TSG level was found to be significantly lower in group2 when compared to group1 and group3 ($p<0.05$).

Group3 and group4 ovarian tissue CAT activity was found to be significantly higher than group1, group2 and group5 ($p<0.05$). When group1, group2 and group5 ovarian tissue CAT activities were compared, no significant difference was found ($p>0.05$). There was no significant difference between group3 and group4 ovarian tissue CAT levels ($p>0.05$).

Table 1. MDA, AOPP, TSG level and CAT activity in Ovarian Tissue in study groups

	Group1	Group2	Group3	Grup4	Group5
MDA (nmol/gr tissue)	$0.39\pm0.15^{b*}$	0.41 ± 0.08^{b}	0.40 ± 0.02^{b}	0.43 ± 0.01^{a}	0.45 ± 0.014^{a}
AOPP (mmol/gr tissue	30.94 ± 1.19^{c}	32.82 ± 0.61^{b}	34.43 ± 0.67^{a}	34.41 ± 0.76^{a}	34.45 ± 0.77^{a}
TSG mmol/gr tissue)	0.59 ± 0.14^{c}	0.55 ± 0.014^{d}	0.58 ± 0.13^{c}	0.62 ± 0.11^{b}	0.66 ± 0.01^{a}
CAT (U/L)	540.38 ± 30.84^{b}	539.51 ± 57.59^{b}	737.12 ± 49.69^{a}	774.56 ± 42.42^{a}	567.81 ± 96.80^{b}

DISCUSSION

ASA is a potent anti-inflammatory medication that inhibits cyclooxygenase. It has been reported that ASA protects endothelial cells from the destructive effect of hydrogen peroxide and has free radical scavenger properties (12). Malondialdehyde (MDA) is a widely used lipid peroxidation product. This aldehyde product is used as an indicator to measure the level of oxidative stress and damage (13). It has been demonstrated that the ovarian tissue MDA level of the group administered 7.5 mg/kg aspirin was not different compared to the control group (3). It has been reported that 30 mg/kg aspirin administration increases MDA levels in the kidney, liver, brain tissues, and plasma (14). Low-dose aspirin leads to peroxidation, and therapeutic-dose aspirin administration has been shown to cause severe peroxidation in erythrocytes and increase the MDA level (15). Group4 and group5 ovarian tissue MDA levels were found to be significantly higher than the other groups ($p<0.05$). In our study, there was no significant difference between group1, group2 and group3 ovarian tissue MDA levels ($p>0.05$). Group 4 and group 5 ovarian tissue MDA levels were similar ($p>0.05$). According to these results, an increase in lipid peroxidation occurs as the aspirin administration dose increases.

Aspirin is considered to have anti-inflammatory properties as it suppresses inflammatory cytokines known to induce oxidative damage in cells. Catalase is an intracellular antioxidant enzyme. Catalase is mainly localized in cellular peroxisomes and to some extent in the cytosol. Catalase catalyzes the reduction of hydrogen peroxide to water and molecular hydrogen (3). Compared to the control group, no significant difference was found in ovarian tissue catalase activity of rats, which were administered 7.5 mg/kg/day aspirin for 15 days (3).

Administration of NSAIDs such as aspirin has been reported to reduce tissue levels of CAT, SOD, and GPx activities (16). NSAIDs are COX enzyme inhibitors. COX is the enzyme that mediates prostaglandin formation from arachidonic acid, and its level is known to increase inflammatory processes (17). It has been demonstrated that catalase activity is elevated in erythrocytes of people receiving aspirin therapy (18). In this study, Group3 and group4 ovarian tissue CAT activity was found to be significantly higher than group1, group2 and group5 ($p<0.05$). When group1, group2 and group5 ovarian tissue CAT activities were compared, no significant difference was found ($p>0.05$). There was no significant difference between group3 and group4 ovarian tissue CAT levels ($p>0.05$).

It has been revealed that anti-inflammatory agents reduce oxidative stress in experimental models involving inflammatory processes. Another remarkable issue to note here is that the suggested antitumor effects of NSAIDs may be associated with the induction of oxidative stress related to numerous signaling pathways, including apoptosis (19).

Advanced oxidation protein products (AOPP) are cross-linked proteins contain dityrosine and are safe markers used to assess oxidative modification of proteins. AOPP is a marker of oxidative stress severity and oxidative-mediated protein damage in inflammation. It is generally produced during oxidative stress or by myeloperoxidase in activated neutrophils through the interaction of hypochlorous acid or chloramines (20, 21). It has been shown that AOPP levels are higher in the serum of women with PCOS (22) and the follicular fluids of women with endometriosis (23). In this study, Group1 (control group) ovarian tissue AOPP level was found to be significantly lower than all aspirin-administered groups ($p<0.05$).

Group2 ovarian tissue AOPP level was found to be significantly lower than group3, group4 and group5 ($p<0.05$).

The balance between oxidants and antioxidants in the organism is the basis for maintaining cellular and biochemical functions. Oxidants damage lipids, proteins, and DNA in cells and even cause death. The most common and rapidly affected proteins are thiols containing sulfhydryl. Plasma thiols are robust antioxidants that remove free radicals from the physiological environment. Plasma thiols serum levels are considered among the markers that indicate antioxidant levels in the body (24). In the presented study, TSG level was found to be significantly higher in group 5 when compared to other groups ($p0<0.05$). Group4 ovarian tissue TSG level was found to be significantly higher when compared to group1, group2 and group3 ($p<0.05$). Ovarian tissue TSG level was found to be significantly lower in group2 when compared to group1 and group3 ($p<0.05$).

CONCLUSION

Aspirin is used in many oxidative stress-related diseases because of its inflammatory and antioxidant properties. Discussions continue about whether the dose of aspirin used should be given as a single dose or in divided doses. In this study, we think that the application of aspirin at intervals of 3 and 5 days may be more effective on the antioxidant system of the ovarian tissue.

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