RESEARCH ARTICLE

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The Therapeutic Efficacy of Gentisic Acid in Torsion-Detorsion Model of Ovarian Tissue in Rats

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Sıçanlarda Yumurtalık Dokusunun Torsiyon-Detorsiyon Modelinde Gentisik Asit'in Terapötik Etkinliği

ABSTRACT

Objective

This study aimed to investigate the therapeutic effectiveness of gentisic acid in ovarian damage caused by torsion-detorsion.

Material and Methods

This research study was conducted using 32 female Wistar rats. All subjects were grouped into sham, torsion detorsion (TD), TD+Low dose and TD+High dose treatment groups in accordance with the experimental procedure. The TD model was created by clamping all of the arteries and collaterals feeding the ovary for 3 hours. Then, the clamp was opened and reperfusion was allowed for 3 hours. In TD+Low and TD+High dose treatment groups, gentisic acid was administered orally at doses of 100 and 200 mg/kg for 1 week, along with the TD model. Anesthesia conditions were created to ensure that the subjects did not feel any pain during all processes.

Results

While the levels of oxidant parameters increased in the TD group compared to the Sham group, antioxidant levels were observed to decrease significantly in the TD+Low dose and TD+High dose groups. It was determined that while the levels of oxidant parameters decreased due to gentisic acid applications, antioxidant levels increased.

Conclusion

Current data showed that ovarian damage caused by TD can be alleviated with low and high dose gentisic acid treatments.

Key Words

Gentisic acid, Torsion detorsion, Ovary, Rat

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ÖZ

Amaç

Bu çalışmada torsiyon detorsiyon aracılığıyla gerçekleşen over hasarında gentisik asit'in tedavi edici etkinliğinin araştırılması hedeflenmiştir.

Gereç ve Yöntemler

Bu araştırma çalışması, 32 adet dişi Wistar sıçan kullanılarak yapıldı. Deney prosedürüne uygun olarak tüm denekler sham, torsiyon detorsiyon (TD), TD+Düşük doz ve TD+Yüksek doz tedavi grupları olacak biçimde gruplandırıldı. TD modeli overi besleyen arter ve kolleterallerin tamamı 3 saat klemplenerek oluşturuldu. Akabinde ise klemp açılarak 3 saat boyunca reperfüzyona izin verildi. TD+Düşük ve TD+Yüksek doz tedavi grupları TD modeli ile beraber 1 hafta süreyle 100 ve 200 mg/kg dozlarında gentisik asit oral yolla uygulandı. Tüm süreçlerde deneklerin acı duymaması için anestezi koşulları oluşturuldu.

Bulgular

Sham grubuna göre TD grubunda oksidan parametrelerin düzeyleri yükselirken, antioksidan düzeylerinin ise TD+Düşük doz ve TD+Yüksek doz gruplarında anlamlı düzeyde azaldığı görüldü. Gentisik asit uygulamalarına bağlı olarak oksidan parametrelerin düzeyleri azalırken, antioksidan seviyelerinin arttığı belirlendi.

Sonuç

Mevcut veriler TD'nin neden olduğu over hasarının düşük ve yüksek doz gentisik asit tedavileri ile hafifletilebileceğini gösterdi.

Anahtar Kelimeler

Gentisik asit, Torsiyon detorsiyon, Yumurtalık, Sıçan

INTRODUCTION

Ovarian torsion is a serious health problem, especially affecting women of reproductive age and resulting in infertility. Ovarian torsion ranks fifth among gynecological emergencies, with an incidence of 2.7%. In ovarian torsion, peritonitis and death may occur following infection (1-4). While surgical intervention is critical in preserving ovarian survival and function in young individuals, ovarian torsion surgeries are generally performed with laparoscopic techniques (4, 5). Detorsion of adnexa following ovarian torsion is important for reducing tissue damage and risk of infertility. Torsion and detorsion (T/D) events together cause ischemia reperfusion (I/R) injury (6, 7).

The intense production of oxygen-derived radicals exacerbates ischemia-based damage by causing peroxidation of many cellular molecules, such as membrane lipids, resulting in direct cytokine production, endothelial damage, and antioxidant enzyme deficiency (8, 9). Some endogenous antioxidant enzymes such as superoxide dismutase (SOD) or some nonenzymatic compounds, such as ascorbic acid, protect the tissue from oxidative damage. Oxidative stress index (OSI), the best indicator of oxidative balance, is calculated by the ratio of total oxidant status (TOS)/total antioxidant status (TAS) (10, 11). In addition to oxidative stress injury, accumulation and activation of neutrophils cause I/R injury. Myeloperoxidase (MPO) levels indicates neutrophil accumulation and activity (12). Increased proinflammatory cytokines have been shown to gradually increase ischemic tissue damage. Interleukin-1 (IL-1) is an important proinflammatory cytokine capable of accelerating the expression of other inflammatory molecules and inducing infiltration and proliferation of neutrophils (13, 14). With all these data, it is noteworthy that antioxidant and anti-inflammatory properties are effective in the I/R model. We have tried to take advantage of these features of gentisic acid. To date, there are various studies evaluating the mechanisms of ovarian T/D-mediated I/R injury. Moreover, studies on this subject have also attempted to repair this tissue and organ damage by using drugs and agents with different biological activities (1, 15, 16).

Non-synthetic products have been the main pharmaceutical ingredients used in the treatment of diseases since ancient times. Moreover, phenolic compounds isolated from natural products and having a wide range of biological activities represent an important type of metabolites that are often studied (17). Gentisic acid (2,5-dihydroxybenzoic acid) is a molecule found in many plants such as melon, gooseberry and is also known as acetylsalicylic acid metabolite (18-20). In addition, scientific studies have reported that gentisic acid shows some pharmacological activities such as anti-inflammatory, antioxidant, neuroprotective and hepatoprotective effects (17, 21). In addition to radical scavenging activities with minimal chelating effect, various medical applications are recommended for gentisic acid (22, 23).

Successful results of gentisic acid in various experimental protocols are available in the literature. However, it has been determined that there is no study investigating the effectiveness of gentisic acid on ovarian tissue has been conducted so far. Therefore, the present study aimed to evaluate the effects of gentisic acid, which possesses various biological properties, on alleviating ovarian oxidative damage in the adnexial T/D model.

MATERIALS and METHODS

Animals and Permissions

The current experiment was approved by the decision dated 2019 and numbered 78 of Atatürk University Experimental Animals Ethics Committee. The study was carried out using healthy Sprague Dawley female rats obtained from the same place in Experimental Animal Research and Application Center of Atatürk University. Rats were kept in the standard laboratory conditions (12 hours in light/dark cycle, 55±5 % humidity and 23±2 °C). Rats had access to food and water ad libitum. To facilitate the sur-

gical process, all animals were deprived of food 12 hours before the experiment, but were allowed to drink water.

Groups and Torsion/Detorsion Model

In the current study, 24 Sprague Dawley female rats (9 weeks old, non-pregnant and weighing 250-270 g) were randomly divided into 4 groups.

The procedure performed in the sham group consisted of only making an abdominal incision and closing it again under anesthesia.

In the T/D group, anesthesia was created and the subjects were fixed in the dorsal position. The abdominal incision area was shaved and cleaned using povidone-iodine solution. Then, a 1-2 cm incision was made. Bilateral arteries, veins of ovary, fallopian tube and ovaries were rotated clockwise by 360 degrees and held with atraumatic microvascular clamp for 3 hours and thus, the torsion process was performed (23). Later, blood circulation was allowed for 3 hours by opening the clamps in the detorsion period. Incision was closed with silk 3/0 suture. The ovarian tissues were removed after 3 h of detorsion.

In low and high dose gentisic acid (GA) groups, gentisic acid (purchased from Sigma Aldrich Co) was administered to animals intraperitoneally at the doses of 100 and 200 mg/kg 30 minutes before reperfusion. Later as described in T/D group, the T/D model was established. The gentisic acid dose used and the ovarian T/D model were applied with reference to previous studies (24, 25).
All surgical procedures applied to animals were performed under anesthesia with 10 mg/kg, i.p xylazine hydrochloride (Rompun®, Bayer, Istanbul) and 60 mg/kg, i.p ketamine (Ketalar®, Pfizer, Istanbul). At the end of the study, animals were decapitated under anesthesia. Ovarian tissues were removed and kept at -80 °C for analysis.

Biochemical Analysis

First, the ovarian tissues taken from the subjects were weighed and homogenized using 2 mL of phosphate buffer. Homogenized tissues were centrifuged at 5000 rpm for 20 min at +4 °C. The supernatants obtained from the homogenate were carefully transferred to microcentrifuge tubes and maintained at -80 °C. The measurement basis of malondialdehyde (MDA, a product of lipid peroxidation) is based on measuring the absorbance at 532 nm of the pink colored compound formed as a result of thiobarbituric acid and MDA reaction (26). TOS and TAS measurements were performed with a commercially available kit (Rel Assay Diagnostics). OSI was accepted as the rate of TOS to TAS. OSI level was calculated as: OSI=[(TOS, µmol/L)/(TAS, mmol/L)×10]. We used OSI as an indicator of oxidative stress. OSI may reflect oxidative status more accurately than TOS. MPO activity determination is based on the absorbance measurement method of the yellowish-orange colored complex at a wavelength of 460 nm, resulting in the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide. The SOD enzyme induces the reaction of the superoxide radical with molecular oxygen to form hydrogen peroxide. In case of insufficient activity of the SOD enzyme, formazan dye is formed as a result of the superoxide and tetrazolium salt reaction. The degree of inhibition of this reaction indicates SOD activity (27, 28). Cytokine levels such as interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) were measured using commercial kits (Elabscience, Wuhan, China).

Table I. Comparisons of Total Antioxidant Status (TAS) (mmol/L), Total Oxidant Status (TOS) (µmol/L) and Oxidative Stress Index (OSI) levels among the experimental groups.

Experimental Groups (n=8)	TAS (mmol/L)	TOS (µmol/L)	OSI
Sham	0.749±0.058	4.828±0.436	0.649±0.093
T/D	0.315±0.049a	8.907±0.967a	2.883±0.527a
T/D+GA 100 mg/kg	0.636±0.075b	5.723±0.685b	0.914±0.172b
T/D+ GA 200 mg/kg	0.735±0.082b	5.408±0.483b	0.745±0.114b

Data are presented as mean±S.D. ap<0.001 compared to control group. bp<0.001 compared to T/D group.

Statistical Analysis

TAS, TOS, OSI, MPO, SOD, TNF-α, IL-1β and MDA results obtained from this study were analyzed using a statistical analysis (SPSS 21, USA) program. Descriptive statistics of the values in the groups were expressed as mean and standard deviation (SD). A p-value < 0.05 was considered statistically significant. One-Way Anova test was performed and Tukey test was used for post hoc binary comparisons. Kruskal Wallis test, which is a non-parametric test, was used for the parameters that do not conform to a normal distribution.

Biochemical Results

At the end of the experiment, the experimental protocol was well tolerated by all experimental animals and no death was observed in the experimental animals. Table I summarizes the ovarian tissue TAS, TOS and OSI values and group comparisons of all groups. Compared with sham group, TAS value was significantly reduced but OSI and TOS increased in T/D group. In therapy groups, TOS, OSI and TAS values were significant between the low-dose gentisic acid group and sham group, but TOS value

was found to be significant between the high-dose gentisic acid and sham group. In both therapy groups, TAS, TOS and OSI values were significantly different compared to the T/D group. A significant difference was found in TAS and OSI values when treatment groups were compared with each other. TOS and OSI values were significantly decreased and TAS values were increase according to T/D group in therapy groups. SOD, MPO, MDA, TNF-α, and IL-1β values were given in ovarian tissue of all groups (Table II and III).

Table II. Comparisons of Superoxide Dismutase (SOD) (U/g protein), Myeloperoxidase (MPO) (U/g protein) activities and Malodialdehyde (MDA) (µmol/g protein) levle among the experimental groups.

Experimental Groups (n=8)	SOD (U/g protein)	MPO (U/g protein)	MDA (µmol/g protein)
Sham	353.15±34.59	297166.67±21463.86	55.98±4.44
T/D	192.75±12.32a	578558.45±28944.04a	94.58±9.28a
T/D+GA 100 mg/kg	295.33±22.54b	321384.54±27540.17b	62.59± 5.40b
T/D+GA 200 mg/kg	373.10±21.26b	290416.02±20156.75b	57.90±4.38b

Data were presented as mean ± S.D. ap<0.001 compared to sham group. bp<0.001 compared to T/D group.

Table III. Comparisons of Tumor Necrosis Factor Alpha (TNF-α) (pg/mg protein), Interleukin 1beta (IL-1β) (pg/mg protein) the experimental groups.

Experimental Groups (n=8)	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)
Sham	14785.99±872.03	19340.72±1549.14
T/D	37633.49±1286.28a	43734.86±3547.99a
T/D+GA 100 mg/kg	17397.73±848.81b	24755.96±1891.43b
T/D+GA 200 mg/kg	15249.13±886.62b	19096.24±1531.77b

Data were presented as mean ± S.D. ap<0.001 compared to sham group. bp<0.001 compared to T/D group.

In the T/D group, all parameters were statistically different from sham group. When the treatment groups were compared to the sham group, there was a statistically significant difference between the tissue concentrations of all parameters except MPO activity in the low dose gentisic acid group. When sham group and high dose gentisic acid group were compared, it was seen that the concentration of all parameters approached those of the sham group and the diffe- rence between the groups could not be determined.

When T/D and both treatment groups were compared, statistical significance was found in all parameters. When the treatment groups were compared with each other, all parameters except MDA were significant. MDA level and MPO activities were significantly decreased and SOD activities were increased according to T/D group in therapy groups. Moreover, a dramatic increase in proinflammatory cytokines was observed in the ovarian T/D group. It was determined that oxidative stress caused by T/D in the tissue triggered proinflammatory cytokines, but cytokine levels were significantly reduced in the low and high dose gentisic acid groups.

DISCUSSION

I/R damage, which occurs with the resumption of blood flow following disruption of tissue vascularization, is important for organ transplantation and graft functions. Prevention of I/R damage is important in recovering cellular functions and preventing necrosis while these cellular and enzymatic events continue to strengthen each other (1, 29). Ovarian torsion is a gynecological emergency, especially seen in the first three decades of life, resulting in ischemia with impaired circulation following bending of the ligaments around the ovary (25, 30). Oxidative damage is the main cause of pathology in ovarian T/D (33).

SOD is common in all cell types and protects the tissue by trying to neutralize the harmful effects of free radicals (10, 12). OSI is a good indicator to show in which direction the oxidative balance is disrupted and helps to understand the extent of oxidative damage (1, 15). Free oxygen radicals resulting from oxidative damage oxidize cell membrane lipids, leading to MDA formation and elevated MDA levels as a marker of lipid peroxidation (5, 25). Proinflammatory cytokines were increased and antiinflammatory cytokines were decreased in rats. MPO is a marker of neu-

trophil infiltration and is one of the major enzymes in I/R. Cytokines, such as TNF-α, IL-1 and IL-6, reactivate the inflammatory cascade through neutrophils and increase damage (31, 32). Inflammation, increased NF-κB expression and inflammatory cytokines such as TNF-α and IL-1β are responsible for tissue damage and are also effective in the pathogenesis and progression of many diseases (33).

Dong et al. also showed in their studies that gentisic acid treatment had therapeutic effects by reducing the serum levels of TNF-α and IL-1β in mice with rheumatoid arthritis (34). In addition to the literature information presented, a previous study found that TNF-α and IL-1β expression was significantly higher in diabetic mice compared to normal control mice, and treatment of diabetic mice with gentisic acid significantly reduced the level of inflammatory cytokines. Additionally, in the same study, TOS and MDA levels increased in diabetic mice. It has been attributed to NF-κB, TNF-α and IL-1β because inflammation is known to increase ROS production in tissues and initiate tissue damage (35). The results of the presented study have documented that gentisic acid application has antioxidant and anti-inflammatory effects, reducing the production of oxidative and inflammatory indices and increasing antioxidant capacity. All these results have been confirmed and supported in previous studies.

There are many studies in the literature that are compatible with the results of this study. In our study, the reduction of oxidant and inflammatory parameters in the ovarian I/R model in rats by gentisic acid suggests that gentisic acid may decrease I/R-induced ovarian injury. Gentisic acid has been shown to have modulatory effects against hepatotoxicity and genotoxicity induced by cyclophosphamide in mice by decreasing MDA and enhancing antioxidant system and possess radical scavenging capacity (36). In another study, gentisic acid was shown to have a beneficial role by increasing antioxidant enzymes, inhibiting tumour promotion responses, and reducing oxidative stress in mice (37). Saeedavi et al., in their study where they created a gentamicin-induced nephrotoxicity model in rats, showed that gentisic acid reduces oxidative stress by showing antioxidant and anti-inflammatory effects (24). Nafees et al. also proved that gentisic acid has a strong antioxidant effect in their studies on genotoxicity and hepatotoxicity caused by cyclophosphamide in mice (36). In another study, they reported that gentisic acid treatment ameliorated type 2 diabetes induced by Nicotinamide-Streptozotocin in male mice by alleviating pancreatic oxidative stress and inflammation through modulation of Nrf2 and NF-κB pathways (38). Within the scope of the study presented in the light of these studies, it was determined that gentisic acid showed antioxidant and anti-inflammatory effects in ovarian tissues. In parallel with these studies, our study showed that gentisic acid has significant antioxidant and anti-inflammatory properties by reducing oxidative markers and pro-inflammatory cytokine levels and increasing antioxidant levels in ovarian tissues. In order to successfully improve the damaging effects caused by I/R, it is essential to better understand the pathophysiology of this damage and develop treatment strategies accordingly.

Clinical and experimental studies on I/R clearly show that suppressing inflammation and oxidative stress developing in the tissue can make tremendous contributions to I/R treatment. Similarly, in this study, the results of gentisic acid in the treatment of I/R are promising, as inflammation and oxidative stress pathways are suppressed by gentisic acid.

CONCLUSIONS

Gentisic acid provides protection against I/R-induced ovarian injury with its antioxidant and anti-inflammatory properties. We have indicated that treatment with gentisic acid reduces ovarian damage in experimental animals exposed to I/R model. Moreover, it has been taken into consideration that further researche is necessary for explaining the other protective mechanism on I/R-induced ovarian injury.

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Ethics Committee Approval
This research complies with all the relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the Experimental Animals Ethics Committee, Atatürk University (approval number: 2019/78).

Informed Consent
All the participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration.

Author Contributions
Concept –A.T, E.P.T.Y, M.C.G, E.E and F.N.E.A; Design - A.T, M.C.G, E.P.T.Y, E.E and FNEA; Supervision - E.P.T.Y, A.T, M.C.G, E.E and FNEA; Resources - E.P.T.Y, A.T, M.C.G and E.E.; Materials - E.P.T.Y, A.T, M.C.G and E.E.;; Data Collection and/or Processing - A.T., M.C.G.; Analysis and/ or Interpretation - E.P.T.Y, A.T, M.C.G and E.E.; Literature Search - M.C.G, A.T. and F.N.E.A; Writing Manuscript - M.C.G, A.T. and F.N.E.A; Critical Review - A.T, E.P.T.Y, M.C.G, E.E and F.N.E.A.

Conflict of Interest
The authors have no conflict of interest to declare.

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