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The effects of acitretin on ovarian reserve: An experimental study in rats

Asitretin'in over rezervi üzerine etkisi: Sıçanlarda deneysel bir çalışma

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Abstract

Background and Design: Acitretin is widely used on many dermatological diseases. No adequate studies have evaluated its effects on ovarian reserve. Thus, this experimental study aimed to investigate the possible effects of acitretin on ovarian reserve by examining ovarian follicle counts and serum anti-Mullerian hormone (AMH) values.
Materials and Methods: This study randomly distributed 31 female Wistar albino rats into 3 groups including one control group (group 1) and two equal experimental groups. Acitretin was given at doses of 0 mg/kg/day (group 1, n=11), 1 mg/kg/day (group 2, n=10), and 5 mg/kg/day (group 3, n=10) for 4 weeks. All the rats in three groups were kept drug-free for 4 weeks before being sacrificed. The blood samples and both ovarian tissues were obtained from the rats. Serum values of AMH were assessed and the number of ovarian follicles was counted by histopathological examination.
Results: The AMH levels were lower in both acitretin groups (groups 2 and 3) than group 1. However, no significant difference was found between the groups in terms of AMH values (p=0.338). Primordial, secondary, tertiary, and total follicle numbers in group 3 were statistically lower than that of groups 1 and 2. The number of primary follicles in group 1 was statistically higher than that of groups 2 and 3. Additionally, the number of secondary follicles in group 2 was statistically higher than that of group 1.
Conclusion: This study showed that ovarian reserve can be affected by acitretin treatment. Particularly, high doses of acitretin may reduce ovarian reserve. Further experimental and clinical studies are needed to clarify the effect of acitretin on ovarian reserve.
Keywords: Acitretin, anti-Mullerian hormone, ovarian reserve

Öz

Amaç: Birçok dermatolojik hastalıkta kullanılmakta olan asitretinin over rezervi üzerine etkisi ile ilgili yeterli çalışma bulunmamaktadır. Bu çalışmada, ratların over rezervi üzerine asitretinin olası etkilerini serum anti-Müllerian hormon (AMH) ve over folikül sayılarını değerlendirerek araştırmayı amaçladık.
Gereç ve Yöntem: Otuz bir dişi Wistar albino sıçan rastgele olarak 3 gruba ayrıldı. Grup 1 (kontrol, n=11) 0 mg/kg/gün, (grup 2, n=10) 1 mg/kg/gün, (grup 3, n=10) 5 mg/kg/gün dozlarında 4 hafta boyunca asitretin verildi. Her 3 gruptaki sıçanlar, 4 haftalık ilaçsız bir dönemi takiben sakrifiye edildi. Sıçanların kan örnekleri ve bilateral over dokuları alındı. Serum AMH değerleri ölçüldü ve histopatolojik olarak over foliküllerinin sayımı yapıldı.
Bulgular: AMH değerleri asitretin gruplarında (grup 2 ve grup 3), grup 1'e göre daha düşük bulundu. Ancak, AMH değerleri açısından gruplar arasında istatistiksel anlamlı fark yoktu (p=0,338). Grup 3'ün primordial folikül, sekonder folikül, tersiyer folikül ve total folikül sayıları, grup

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1 ve grup 2'ye göre istatistiksel olarak daha düşüktü. Grup 1'deki primer folikül sayısı hem grup 2'den hem de grup 3'den daha yüksekti ve fark istatistiksel olarak anlamlıydı. Ayrıca, grup 2'nin sekonder folikül sayısının grup 1'den anlamlı düzeyde yüksek olduğu saptandı.
Sonuç: Bu çalışma, over rezervinin asitretin tedavisinden etkilenebileceğini göstermektedir. Özellikle asitretin yüksek dozları over rezervinin azalmasına neden olabilir. Asitretinin over rezervi üzerindeki etkisini açıklığa kavuşturmak için daha fazla deneysel ve klinik çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Asitretin, anti-Müllerian hormon, over rezervi

Introduction

Acitretin is a second-generation retinoid and a pharmacologically active metabolite of etretinate[1,2]. It replaced etretinate due to its more favorable pharmacokinetic profile. Its mechanism of action is not well known; however, acitretin has a wide variety of biological functions[1,3].
Acitretin is widely used in various dermatological diseases, such as psoriasis, lichen planus, Darier's disease, hidradenitis suppurativa, pityriasis rubra pilaris, ichthyosis, and keratodermas. The main side effects of acitretin are well known. Additionally, acitretin is also known as teratogenic regardless of dosage or treatment duration. Therefore, the use of two effective forms of contraceptives is recommended during the treatment period and for 3 years after therapy discontinuation[1]. Studies did not show any negative effects of acitretin on spermatogenesis, sperm morphology, sperm motility, or reproductive hormones[4-6]. However, a study that explores the effects of acitretin on ovarian reserve after therapy cessation has not been previously reported. Such that, female patients who will take acitretin may worry about infertility and ovarian reserve after acitretin cessation.
Anti-Mullerian hormone (AMH) reflects ovarian function better than serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B, and estradiol (E_2). Therefore, AMH has recently been used to determine ovarian reserve, which is associated with the fertility potential of females. Serum AMH values were more strongly correlated with antral follicle count (AFC)[7,8]. Although it is not an excellent test for ovarian reserve evaluation, both AFC and AMH have well predictive value[9].
The potential side effects of acitretin on ovarian reserve are unknown. Thus this study aimed to experimentally determine the effect of acitretin on ovarian reserve by evaluating ovarian follicle counts and serum AMH values.

Materials and Methods

Animals and experimental design

Study approval was obtained from Aydın Adnan Menderes University Animal Experiments Local Ethics Committee for this animal experiment (approval number: 64583101/2016/009). From the Experimental Animal Production and Research Laboratory of Aydın Adnan Menderes University, 4-6-month-old 31 female Wistar rats were taken. All experiments were conducted following the principles and guidelines approved by the Aydın Adnan Menderes University Animal Ethical Committee.
All animals were provided with commercial rat feed and water. They were maintained in a 12-hour light/dark cycle at a temperature of 24±2 °C.
Thirty-one female Wistar albino rats were randomly distributed into 3 groups including one control group (group 1) and two equal experimental groups. The groups were explained as follows:
Group 1 (n=11) was designed as a control group, wherein the rats were not administered any medication.
The rats in group 2 (n=10) were administered 1 mg/kg/day of oral acitretin for 4 weeks. The rats in group 3 (n=10) were administered 5 mg/kg/day of oral acitretin for 4 weeks.
Higher doses of acitretin were preferred to use in rats compared to the doses used in humans since the drug metabolism in rats is higher than in humans. Additionally, acitretin has been used in high doses in previous experimental rat studies[4,10,11].
Every Monday, all rats were weighed to adjust the doses of acitretin. The capsule form of acitretin (Neotigason® 10 mg capsule, Actavis, İstanbul, Turkey) was diluted with 1 mL of distilled water and administered daily to the rats in experimental groups according to their weight. All dilutions were prepared daily for 4 weeks. Thereafter, neither the rats in the experiment groups nor the rats in the control group received any treatment for 4 weeks (drug-free period).
At the end of 8 weeks, the blood samples of all rats were obtained by cardiac puncture under the anesthesia of xylazine (5 mg/kg) and ketamine (50 mg/kg). Then, the rats were sacrificed, and bilateral ovariectomy was performed. Both ovarian tissues were fixed in 10% formalin solution. Additionally, blood samples were centrifuged at 3000 rpm. Then the sera were stored at -80 °C for AMH measurement.

Histopathological follicle evaluation and counting

Ovarian tissue samples were fixed in formaldehyde (10%) solution. After 1 day, the tissues were fixed in paraffin. Subsequently, samples were cut in 5 µm sections with a microtome and placed on the slides. After deparaffinization and rehydration, the slides were stained with hematoxylin and eosin. All slides were inspected by a pathologist who is blind to the groups. All sections were analyzed using a light microscope. Five sections at various levels were taken from both ovaries. Follicles containing oocytes were classified according to their developmental stages based on previous studies[12,13]. Later, follicles were identified as primordial, primary, secondary, or tertiary (antral). Primordial follicle is defined with the monolayer squamous (flat) granulosa cells that partially or completely surround the oocyte. The primary follicle is defined with a central oocyte surrounded by single-layer cuboidal granulosa cells. The secondary follicle contains an oocyte surrounded by a multilayer of granulosa cells without antrum formation. Finally, the tertiary (antral) follicle includes an oocyte surrounded by multilayers of granulosa cells with antrum formation.

Measurement of serum AMH

AMH serum values of the rats were measured using a commercial enzyme-linked immunoabsorbent assay kit (rat ELISA kit, Sunred, Baoshan District, Shanghai, China). For AMH, the kit sensitivity was 0.101 ng/mL with a coefficient of variation of <5%. Procedures were conducted following the manufacturer's instructions. AMH serum levels of 8 serum samples that are randomly selected from each group were measured.

Statistical Analysis

The Statistical Package for the Social Sciences version 20 software program was used for statistical analysis. The mean ± standard deviation, frequency, and percentage values were used for the variables. Additionally, homogeneity of variances from prerequisites of parametric tests was checked using Levene's test. The normality hypothesis was examined using the Shapiro-Wilk test. When the normality assumption was provided, the One-Way analysis of variance for the comparison of three or more groups and the Tukey HSD test, a multiple comparison test, were used. When not provided, Kruskal-Wallis and Bonferroni-Dunn, which is also a multiple comparison test, were used. P-values of $p<0.01$ and $p<0.05$ were accepted as statistically significant for data analysis.

Results

The serum AMH levels were lower in both experimental groups 2 and 3 (4.2±0.77 and 3.62±0.98, respectively) compared to group 1 (4.39±1.34). However, no statistically significant difference was found among the groups for serum AMH levels (p=0.338) (Table 1).

The counts of primordial, secondary, and tertiary follicles were significantly lower in group 3 compared to groups 1 and 2. Additionally, the total number of follicles was significantly lower in group 3 compared to groups 1 and 2. Statistically significant differences were not found between groups 1 and 2 in the number of primordial, tertiary, and total follicles. The number of primary follicles significantly decreased in groups 2 and 3 compared to group 1. Additionally, the number of secondary follicles was significantly higher in group 2 than that in group 1 (Table 2).

Discussion

Changes in the pituitary hormones, such as FSH and LH, have been evaluated in patients using acitretin in previous studies[14,15]. A study that involve 43 patients with psoriasis (24 males and 19 females) reported no significant changes in FSH, LH, E_2, and total testosterone values before and after acitretin treatment[14]. A study that was conducted with male patients with psoriasis revealed no significant alterations in FSH, LH, and testosterone levels during the acitretin treatment compared to pretreatment values[15]. Another study on males who are treated with acitretin observed that acitretin in therapeutically effective doses did not influence spermatogenesis, sperm motility, sperm morphology, and hypothalamic-pituitary-gonadal axis[5].

AMH is a glycoprotein that is expressed by granulosa cells of developing follicles in the ovary and participates in folliculogenesis regulation[16]. AMH is more powerfully associated with ovarian follicular status than FSH, LH, inhibin B, and E_2[7,8]. Additionally, serum AMH levels quantitatively assess ovarian damage caused by ovarian toxic interventions, such as chemotherapy, instead of qualitative evaluation using menstrual cycle or basal FSH values[17]. No test is excellent; however, both AFC and AMH have good predictive values for ovarian reserve evaluation[9]. Our study revealed lower serum AMH values in acitretin groups compared to the control group, although it was not statistically significant. There was a significant decrease in the number of all follicle types in group 3 compared to the control group. These findings suggest that higher doses of acitretin may have detrimental effects on the ovaries. In the light of these results, the use of acitretin at higher doses is thought to decrease ovarian reserve, at least in the early period. Furthermore, the significantly decreased number of primary follicles in group 2 compared to group 1 suggests that acitretin may have a negative effect on ovarian reserve. The reason for the higher number of secondary follicles in group 2 compared to group 1 could not be understood.

In the literature, studies investigated the effects of isotretinoin, which is another commonly used retinoid, agent on ovarian reserve and female fertility. A study with rats revealed that isotretinoin exposure may be responsible for reduced ovarian reserve and harmful effects on rat ovaries[18]. A study of females with acne revealed reduced AMH values following an isotretinoin exposure, which may suggest that isotretinoin has a harmful effect on the ovaries[19]. Another study with females of reproductive age who are treated with oral isotretinoin for acne, revealed decreases in serum AMH, total AFC, and ovarian volume (OV) following isotretinoin treatment and concluded that isotretinoin treatment had a significant unfavorable effect on the ovarian reserve[20]. A study on the long-term effect of systemic isotretinoin on female fertility revealed that the AMH, OV, and AFC levels at 6 months (at the end of isotretinoin treatment) were significantly lower compared to the levels at 18 months (following a 12-month treatment-free period). However, AMH, OV, and AFC levels at the beginning of treatment and

Table 1. Serum AMH values of all groups

	Group 1 (control) (n=8)	Group 2 (1 mg/kg/day acitretin) (n=8)	Group 3 (5 mg/kg/day acitretin) (n=8)	p
AMH	4.39±1.34	4.2±0.77	3.62±0.98	0.338

AMH: Anti-Mullerian hormone, all values are expressed as mean ± standard deviation, $p<0.05$ was accepted as statistically significant

Table 2. Follicle numbers of all groups

	Group 1 (n=11)	Group 2 (n=10)	Group 3 (n=10)	p
Primordial follicle	62.82±9.87[b]	61.4±11.38[b]	49±7.73	0.006[**†]
Primary follicle	55.27±9.05[a,b]	49.1±11.06	40.9±5.43	0.004[** †]
Secondary follicle	47.91±6.74[a,b]	53.1±13.23[b]	40.5±3.84	0.012[*‡]
Tertiary follicle	15.82±3.84[b]	14±3.13[b]	9.2±1.62	0.001[**‡]
Total follicle	181.82±22.35[b]	177.6±29.72[b]	139.6±16.28	0.001[*†]

All values are expressed as mean ± standard deviation, [a]: Statistically different from group 2, [b]: Statistically different from group 3, ‡: One-Way ANOVA test or Tukey HSD test, †: Kruskal-Wallis test or Bonferroni-Dunn test, $p<0.05$ and $p<0.01$ ([*]: $p<0.05$, [**]: $p<0.01$) were accepted as statistically significant

18 months were statistically similar. Ultimately, the authors suggested that the damaging effects of systemic isotretinoin therapy on ovarian reserve have vanished over time[21]. Therefore, we think that the long-term effect of acitretin on ovarian reserve should be further examined.

Study Limitations

The first limitation of our study is the evaluation of ovarian reserve only by serum AMH values and the number of ovarian follicles. The second limitation is its experimental study design using rats as a model and animal studies are known to not always mimic the outcome of human studies. We believe that more precise information can be reached on this matter with human subjects or different experimental designs.

Conclusion

Results of this experimental study indicate that acitretin may display negative effects on ovarian reserve. It is not possible to conclude with this experimental study whether acitretin will have a negative effect on the ovarian reserve of women in the period when the pregnancy is allowed, which is three years after the cessation of the treatment. Finally, we think that randomized and controlled animal-human studies are needed to receive precise results regarding the acute and long-term effect of acitretin on ovarian reserve.

Ethics

Ethics Committee Approval: Study approval was obtained from Aydın Adnan Menderes University Animal Experiments Local Ethics Committee for this animal experiment (approval number: 64583101/2016/009).
Informed Consent: Patient approval has not been obtained as it is performed on animals.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.G., B.D., S.Y.Ç., M.Y., Concept: M.G., Design: M.G., B.D., Data Collection or Processing: M.G., B.D., S.Y.Ç., M.Y., Analysis or Interpretation: M.G., B.D., T.A., Literature Search: M.G., Writing: M.G., B.D., T.A.
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References

1. Sarkar R, Chugh S, GargVK: Acitretin in dermatology. Indian J Dermatol Venereol Leprol 2013;79:759-71.
2. Khalil S, Bardawil T, Stephan C, et al.: Retinoids: A journey from the molecular structures and mechanisms of action to clinical uses in dermatology and adverse effects. J Dermatolog Treat 2017;28:684-96.
3. Guenther LC, Kunynetz R, Lynde CW, et al.: Acitretin use in dermatology. J Cutan Med Surg 2017;21:2S-12.
4. Sengör B, Bayramgürler D, Müezzinoglu B, Altintas L, Bilen N, Apaydin R: Effects of acitretin on spermatogenesis of rats. J Eur Acad Dermatol Venereol 2006;20:689-92.
5. Parsch EM, Ruzicka T, Przybilla B, Schill WB: Andrological investigations in men treated with acitretin (Ro 10-1670). Andrologia 1990;22:479-82.
6. Yiu ZZ, Warren RB, Mrowietz U, Griffiths CE: Safety of conventional systemic therapies for psoriasis on reproductive potential and outcomes. J Dermatolog Treat 2015;26:329-34.
7. Dayal M, Sagar S, Chaurasia A, Singh U: Anti-Mullerian hormone: A new marker for ovarian function. J Obstet Gynaecol India 2014;64:130-3.
8. Fanchin R, Schonäuer LM, Righini C, Guibourdenche J, Frydman R, Taieb J: Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod 2003;18:323-7.
9. Tal R, Seifer DB: Ovarian reserve testing: A user's guide. Am J Obstet Gynecol 2017;217:129-40.
10. Czerny B, Teister M, Juzyszyn Z, et al.: The effect of retinoic acid receptor agonist acitretin on the production of bile and concentrations of some serum components in ovariectomized rats. Menopause 2011;18:213-8.
11. Tsambaos D, Bolsen K, Georgiou S, Kalofoutis A, Goerz G: Effects of oral administration of acitretin on rat liver microsomal phospholipids, P-450 content and monooxygenase activities. Skin Pharmacol 1994;7:320-3.
12. Myers M, Britt KL, Wreford NG, Ebling FJ, Kerr JB: Methods for quantifying follicular numbers within the mouse ovary. Reproduction 2004;127:569-80.
13. Xu CK, Zhao YH: Apoptosis of rat's ovarian follicle cells induced by triptolide in vivo. Afr J Pharm Pharmacol 2010;4:422-30.
14. Karadag AS, Ozlu E, Kostek O, Bilgili SG, Balaharoglu R, Ertugrul DT: Effect of low dose acitretin treatment on pituitary h hormones in psoriasis vulgaris: A retrospective study. Indian J Dermatol Venereol Leprol 2019;85:300-4.
15. Angioni AR, Lania A, Cattaneo A, Beck-Peccoz P, Spada A: Effects of chronic retinoid administration on pituitary function. J Endocrinol Invest 2005;28:961-4.
16. Zec I, Tislaric-Medenjak D, Megla ZB, Kucak I: Anti-Mullerian hormone: A unique biochemical marker of gonadal development and fertility in humans. Biochem Med (Zagreb) 2011;21:219-30.
17. Iwase A, Nakamura T, Nakahara T, Goto M, Kikkawa F: Anti-Mullerian hormone and assessment of ovarian reserve after ovarian toxic treatment: a systematic narrative review. Reprod Sci 2015;22:519-26.
18. Abali R, Yuksel MA, Aktas C, et al.: Decreased ovarian reserve in female Sprague-Dawley rats induced by isotretinoin (retinoic acid) exposure. Reprod Biomed Online 2013; 27:184-91.
19. Sikar Aktürk A, Abalı R, Yüksel MA, Çelik Güzel E, Güzel S, Kıran R: The effects of isotretinoin on the ovarian reserve of females with acne. Gynecol Endocrinol 2014;30:30-3.
20. Aksoy H, Cinar L, Acmaz G, et al.: The effect of isotretinoin on ovarian reserve based on hormonal parameters, ovarian volume, and antral follicle count in women with acne. Gynecol Obstet Invest 2015;79:78-82.
21. Cinar SL, Kartal D, Aksoy H, et al.: Long-term effect of systemic isotretinoin on female fertility. Cutan Ocul Toxicol 2017;36:132-4.