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ARTICLE

Decreased ovarian reserve in female Sprague–Dawley rats induced by isotretinoin (retinoic acid) exposure

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Abstract Isotretinoin is a retinoid widely used for the treatment of severe nodulocystic acne. Although it has broad side effects, there is no well-designed study about its effects on the ovary. This study investigated possible toxic effects of isotretinoin on female gonads. A total of 30 female rats were randomly divided into three equal groups according to the dose of isotretinoin they were administered: 0 mg/kg/day (group 1), 7.5 mg/kg/day (group 2) or 15 mg/kg/day (group 3). Thirty days after the treatment, the effects of isotretinoin on the ovaries were evaluated with serum anti-Müllerian hormone (AMH) concentrations, apoptosis by TUNEL assay and immunohistochemical observations by proliferating cell nuclear antigen (PCNA). The percentage of atretic follicles was calculated for each stage of folliculogenesis. The serum AMH concentrations were found to be lower in both isotretinoin groups. The percentage of atretic follicles in both isotretinoin groups was higher than the control. The number of PCNA-positive granulosa cells was decreased in the isotretinoin groups. The number of ovarian follicles with apoptotic granulosa cells was increased in the experimental groups. These data are the first to identify that exposure of isotretinoin may be responsible for decreased ovarian reserve and toxic effects on rat ovaries.

KEYWORDS: anti-Müllerian hormone, apoptosis, isotretinoin, ovarian reserve, toxicity, atretic follicle

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Introduction

Isotretinoin (13-*cis*-retinoic acid) is a retinoid that has been widely used for the treatment of severe recalcitrant nodulocystic acne since 1982 (Jones et al., 1980). The exact mechanism of retinoid action is not clear. There are numerous studies indicating the modes of action of isotretinoin, which include the induction of apoptosis and cell cycle arrest in human sebaceous gland cells (SEB-1 sebocytes) near increased TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) staining of treated cells (SEB-1 sebocytes) and increased concentrations of cleaved caspase 3 (Nelson et al., 2006).

The physiological functions of retinoids include the control of proliferation, apoptosis and differentiation in normal cells during growth and development; they also have tumour-suppressive capacity (Altucci and Gronemeyer, 2001).

Isotretinoin for acne treatment is used for approximately 6 months at a dose of 0.5—1 mg/kg/day to a cumulative dose of 120—150 mg/kg. However, there is emerging evidence that much lower dosages (as low as 5 mg/day) are just as effective but have significantly fewer adverse effects (Sardana and Garg, 2010). It has been used not only for severe acne vulgaris but also for the management of other dermatological conditions, such as rosacea, folliculitis, sarcoidosis, granuloma annulare, seborrhoeic dermatitis, myelodysplastic syndromes, chemoprevention of skin neoplasms, periorificial dermatitis and a variety of disorders of keratinization (Akyol and Ozcelik, 2005; Brelsford and Beute, 2008).

Isotretinoin has a broad spectrum of side effects. The most common side effects occur in the mucocutaneous and ocular regions. Yet the most notable side effect is the induction of birth defects due to fetal exposure to isotretinoin, which is pregnancy category X (Tzimas and Nau, 2001). Therefore, contraception is necessary during isotretinoin treatment in women of childbearing age, beginning 1 month before, during and 3 months after treatment.

Acne vulgaris often affects young people between 12 and 18 years of age, some of whom are treated with isotretinoin. While this drug is known to have many side effects, there are only a few studies describing the effects on reproductive organs (Comitato et al., 2006; Ferguson et al., 2005; Gencoglan and Tosun, 2011).

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a dimeric ovarian glycoprotein produced by the granulosa cells of healthy, small, growing follicles (Lee and Donahoe, 1993; Themmen, 2005). AMH expression disappears when a follicle becomes atretic, and it is also a reliable marker of ovarian reserve, with decreasing concentrations correlated with reduced response potential (Fleming et al., 2013; Nelson and La Marca, 2011). Recent studies suggest that serum AMH may be useful as a biomarker for ovarian reserve following chemotherapy and exposure to other gonadotoxic agents (Anders et al., 2008; Anderson and Cameron, 2011; Lie Fong et al., 2008; van Beek et al., 2007). AMH is an earlier predictor of ovarian reserve in the ageing process compared with other identified ovarian hormones, such as FSH, inhibin B or oestradiol and does not vary significantly across the menstrual cycle (Anderson, 2012).

This study postulated that, in isotretinoin-exposed female rats, decreasing concentrations of serum AMH could be a sign of ovarian damage and that isotretinoin causes damage to the ovary by increasing the percentage of atretic follicles. As far as is known, there is no study in the literature investigating the effects of isotretinoin treatment on female rat ovaries.

Materials and methods

Animals

Female, 7—8-week-old, Sprague—Dawley rats were obtained from the Institute of Experimental Medicine, Istanbul University. The animals were maintained on a 12/12 light/dark cycle with ad-libitum access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Istanbul University.

Experimental design

A total of 30 female rats were randomly divided into three experimental groups according to the dose of isotretinoin to be administered: 0 mg/kg/day (group 1), 7.5 mg/kg/day (group 2) or 15 mg/kg/day (group 3). Each group was comprised of 10 animals.

Drug administration

Capsules of isotretinoin (Zoretanin; Actavis Laboratories) were opened and transferred to class A volumetric flasks and diluted with soybean oil (cat. no. 8001-22-7; MP Biomedicals) to obtain suspension at the desired concentrations. All drug preparations were conducted in a darkened room and amber bottles were used for storage. Rats were gavaged once daily with either 0 (soybean oil only), 7.5 or 15 mg/kg/day in a volume of 1.42 ml/kg. Daily gavages continued for 30 consecutive days. The doses of 7.5 or 15 mg/kg/day were given to produce serum isotretinoin concentrations comparable to those of humans treated with 1 mg/kg/day based on the results of a previously published study (Ferguson et al., 2006). Serum isotretinion concentrations were not measured in the current study.

Histopathological evaluation

At the end of the experiment, the ovaries were individually immersed in Bouin's fixative, dehydrated in alcohol and embedded in paraffin. Serial sections of 5 μm were obtained, deparaffinized and stained with haematoxylin—eosin (H and E). Analysis of normal and atretic primordial, primary and preantral follicles was performed in the largest cross-section of each ovary. The total number of normal and atretic follicles per ovary was calculated, and the percentage of atretic follicles was calculated as 100 × atretic follicles total follicles (Zhang et al., 2006).

Classification of follicles

Follicles were histologically classified as normal or atretic. Normal follicles had an intact granulosa layer with a