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Original Article

Investigation of genotoxicity caused by oral isotretinoin use in acne treatment

Ebru Derici Eker, Serkar Kocoglu, Nefise Ozlen Sahin

Mersin University Pharmacy Faculty Department of Pharmaceutical Biotechnology, Mersin, Turkey

Abstract

Aim: Acne vulgaris is an inflammatory disease with a multifactorial origin in the pilosebaceous unit affecting approximately 85% of the population aged 11-23 years. Although it does not present a life-threatening condition, it can cause social phobia and depression due to its psychological and physical effects. Isotretinoin is one of vitamin A-derived retinoids and has been used in moderate and severe acne cases for more than 25 years. Genotoxicity is an expression covering the damage caused by genotoxic substances in DNA structure and chromosomes.

Material and Methods: The aim of this study was to investigate whether isotretinoin used orally in the treatment of acne vulgaris has an effect on micronucleus frequency which is one of the most important biomarkers of genotoxicity. The experimantal group consisted of 30 women aged 20-23 years who were diagnosed with acne and used isotretinoin for at least 3 months, and the control group consisted of individuals who did not use isoretinoin with similar properties. Buccal smear samples were taken from all groups and micronucleus test was performed.

Results: In the experimental group, the micronucleus frequency was 109.4±13.84. In the control group, the micronucleus frequency was 96.2±10.13. The difference between the experimental and control groups in terms of micronucleus frequency was found to be statistically significant (p=0.026).

Conclusions: As a result, it is emphasized that isotretinoin indication should be done more carefully in the treatment of acne and it is very important for the patient, relatives and public health.

Keywords: Acne; isotretinoin; genotoxicity; micronucleus test.

INTRODUCTION

Acne is a chronic inflammatory disease of multifactorial origin that occurs in the hair follicle and follicle-bound sebaceous glands (1). It usually starts around puberty (2). Continues for a while then shows a tendency to spontaneous recovery. In some cases, recovery may be delayed until the age of 30-35 (3). It is mainly seen in adolescence, but it can also be seen during periods when sebaceous glands such as neonatal, infantile, prepubertal and adult are active (4,5). Acne was observed more frequently in girls between the ages of 13-16 and in boys between the ages of 15-18 (1,4). In addition, acne is reported to be more frequent and more severe in men than in women (6). Approximately 80% of the population has to live with acne vulgaris at some time in their lives (7). Although it is not a life-threatening situation, it can cause social phobia with its physical and psychological effects especially during adolescence (8).

The genetic predisposition of acne has also been shown in several studies. Acne vulgaris was found in 45% of children with acne vulgaris in one or both of their parents, and in 8% of children without acne vulgaris. In monozygotic twins, the severity of acne vulgaris, sebum release rate and comedon count were similar (9). Since most of the patients with acne have normal androgen levels in the serum, it is thought that a change in genetically androgen receptor or 5α-reductase enzyme level or activity may cause acne. In addition, studies have shown that androgen receptor gene is present in the q11-12 region of the X chromosome and its activation varies according to age, sex, and genetic characteristics (10).

There is no standard treatment regimen for the treatment of acne vulgaris (9). The use of topical and systemic agents, combined treatment and surgical treatment options are available (11). The treatment to be applied to the patient is determined according to the results of the detailed

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Corresponding Author: Ebru Derici Eker, Mersin University Pharmacy Faculty Department of Pharmaceutical Biotechnology, Mersin, Turkey, **E-mail:** edeker@mersin.edu.tr

examination of the patient. Detailed history should be taken including the patient's sex, age, occupation, lifestyle, previous treatments, corticosteroid, oral contraceptive and anabolic steroid medications and concomitant diseases. In addition, a detailed physical examination is performed and laboratory tests are reviewed if necessary. Accompanying all this information, acne severity, type of lesion, psychological effects of the disease and the treatment scheme to be applied according to the source of the disease emerges (9).

The goal of treatment is to reduce the number and severity of the lesion and to prevent scar development. Generally, topical treatments are sufficient in patients with mild acne, while combined use of topical and systemic treatments is required in patients with moderate to severe lesions (12). Early treatment of acne is recommended to prevent scar tissue formation (13).

Isotretinoin is a stereoisomer of tretinoin (retinoic acid) in the all-trans position. Systemic isotretinoin has been used in the treatment of acne since 1982. Increased sebum production, ductal hypercornification, P. acnes colonization and inflammation are the four main factors that play a role in the etiology of acne. The details of the mechanism of action are not fully known (14,15). However, retinoid act on two distinct nuclear receptor groups, which are characterized as RAR and retinoic X receptors (RXR). These receptors bind to target sequences on DNA to activate gene transcription (16).

Endogenous retinoid show their biological effects through DNA transcription through retinoic acid receptors in the cell nucleus. Isotretinoin does not bind directly to retinoic acid receptors, acts as a prodrug and binds to the retinoic acid receptor after it is converted to its active form in the sebocyte. Isotretinoin reduces comedon formation by normalizing keratinocyte maturation and adhesion. It has also been found to reduce sebocyte-derived androgen synthesis and sebum synthesis by 80% in the first month of treatment (9). Isotretionin acts by suppressing sebaceous gland activity and histologically shrinking the sebaceous glands, as well as being dermally anti-inflammatory. The use of isotretinoin in pregnancy is absolutely contraindicated. Contraception is compulsory, starting from one month before isotretinoin treatment during the treatment and up to one month after discontinuation of treatment (1,9,13,15,17).

Standard isotretinoin treatment duration is 16-20 weeks. Approximately 85% of patients achieve remission in 16 weeks, while 15% require longer-term treatment. Isotretinoin, administered at a daily dose of 1 mg / kg, reduces sebum production by about 10% compared to the pre-treatment period and reduces the sebaceous glands, with maximum inhibition occurring at 4 or 5 weeks. In the first month, superficial lesions such as papules and pustules are regressed (9). In order to observe a significant decrease in the number of cysts, a treatment period of at least 8 weeks is required. In recent years, new isotretinoin formulations and low-dose or intermittent treatment protocols have been tried (9,16). It has been reported that low-dose or intermittent treatment protocols, such as 0.1 mg / kg / day, may also be applied, especially in adult patients, oily skin, and chronic mild to moderate acne (9).

Genetic toxicity or genotoxicity is a term covering the damage caused by genotoxin substances in chromosome and DNA structure. These damages are usually gene mutations, chromosomal abnormalities, DNA insertions and DNA chain breaks. Genotoxic effect is defined as the genotoxic substances that interact with enzymes that provide replication of DNA or genome and cause mutations or cause some changes in DNA. Since such genetic damage can lead to birth defects, cancer, infertility, aging, and some genetic and multifactorial diseases, it is important to define and minimize the risk of mutagen and carcinogens in order to maintain health (18).

Genetic toxicity tests are used to determine the mutagenicity of chemical and physical agents and to predict their carcinogenic potential. These are bio-monitoring tests that enable the investigation of the genotoxic and carcinogenic potentials and safety of all kinds of chemicals that we are constantly exposed to in our daily lives such as drugs, physical agents, food additives and environmental pollutants, estimating cancer risk and monitoring cancer. These tests consist of in vitro and in vivo tests developed to detect damage directly or indirectly to the genetic structure. However, it is emphasized that a test alone is not sufficient to determine the genotoxic effects of the substances, therefore a series of test systems should be used to determine the genotoxic or mutagenic activity of the compounds (18,19).

In vitro and in vivo mutagenicity tests that provide a link between the carcinogenic and mutagenic potentials of the substances whose genotoxicity we want to detect; Ames test, Comet test, Chromosomal abnormalities (CA) test, sister chromatid exchange (SCE) test and Micronucleus (MN) test (18).

Micronuclei are small extracellular bodies that occur during metaphase / anaphase transition when the cell is in mitosis. They are defined as small spherical structures outside the main nucleus within the cell cytoplasm, but with the same structure, shape and staining (19). DNA damage is the basis of MN formation. Exposure of the organism to various mutagenic, clastogenic and carcinogenic agents results in DNA destruction (18,19,20). The importance of MN test in toxicology, pharmaceutical industry, diet, suspicion of cancer risk and radiotherapy have been understood in genetic damage measurement. It has become one of the most economical and practical techniques for determining DNA damage rate in vivo and in vitro.

Micronucleus technique is used in the analysis of genotoxic effects of chemical agents in human peripheral lymphocytes, bone marrow and buccal mucosa cells. This technique is important because it is easy to use, it gives

fast results and it is reliable in determining DNA damage. It is now known that there is a link between many types of cancer and specific chromosomal disorders. For this reason, micronucleus test is performed in cancer patients and significant results are obtained (20).

MATERIAL and METHODS

The study group consisted of 30 women aged 20-23 years who were diagnosed with acne and used isotretinoin for at least 3 months. The control group consisted of 30 female subjects between the ages of 20 and 23, who had not used isotretinoin before and who had not used any drugs and who had no smoking and alcohol habits. All individuals were asked to complete the informed consent form within the framework of ethical rules and detailed information was given about the study. Then buccal smear samples were taken from these individuals and necessary analyzes were performed.

For micronucleus (MN) test; after each individual was asked to rinse their mouths, buccal smear samples were taken from the inner mucosa of the mouth with a wooden spatula and spread on slides and dried at room temperature. It was then fixed in cold methanol (Merck 1060082500) for 12 hours. After fixation, air-dried preparations were stained in 5% Giemsa dye solution (Merck 1092040500) for 10 minutes. Preparations washed under running water were dried and made ready for microscopic examination. Preparations were examined with a 40X objective under a binocular light microscope (Nikon Eclipse E200, Japan). During these examinations, 1000 epithelial cells were counted for each individual and total micronucleus frequency (% MN) was determined by recording the number of micronucleus observed in these cells. Mann-Whitney U test was used to determine whether there was a statistically significant difference between the experimental and control groups. SPSS for Windows v16 package program was used in these analyzes.

RESULTS

The aim of this study was to investigate whether isotretinoin, commonly used among young people, has an effect on micronucleus (MN) frequency, one of the most widely used biological markers of genetic toxicity. For this purpose, the control group of our study consisted of 30 healthy female individuals, and the experimental group consisted of 30 female individuals who were diagnosed with acne and used isotretinoin for at least 3 months.

The mean age of the control group was 22.3±0.73 years and the mean age of the experimental group was 22.1±0.67 years. There was no statistically significant difference between the experimental and control groups in terms of age (p=0.534).When we examined the MN frequency between the experimental and control groups, the MN frequency in the experimental group was 109.4±13.84 and the MN frequency in the control group was 96.2±10.13. The difference between the experimental and control groups in terms of MN frequency was found to be statistically significant and the results are shown in Table 1 (p=0.026). MN frequency of experimental and control groups is shown in the graph in Figure 1.

Table 1. MN distribution in experimental and control groups

Group	Average MN Frequency	Number of People	Standard Deviation	Minimum MN Value	Maximum MN Value	Average MN Value
Experimental Group	109.4	30	13.84197	87.00	128.00	111.5000
Control Group	96.2	30	10.13026	86.00	118.00	94.5000
Total	102.8	60	13.60959	86.00	128.00	98.5000

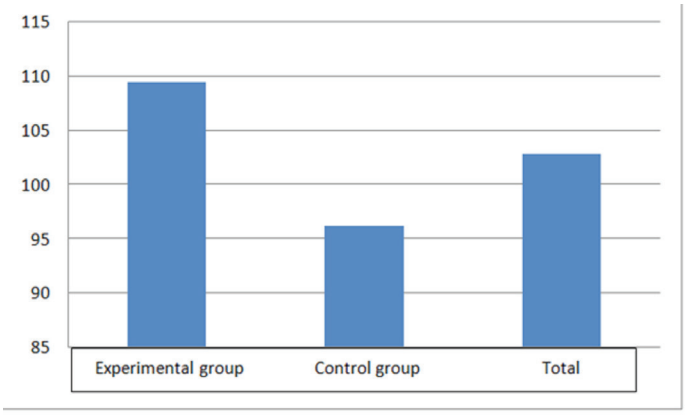

Figure 1. MN Frequency distribution in all groups

DISCUSSION

In our study, we investigated the difference between the frequency of MN formation in patients and controls in patients using isotretinoin in order to investigate the genotoxicity of acne drugs. In our study, the mean age, sex, and duration of isotretinoin use were evaluated in all patients and the data we obtained were generally similar to those in the literature.

In a study conducted by Yıldırım et al. in the printing industry, MN and control groups were compared for all experimental and control groups; MN percentage of the experimental group was 0.29±0.16, and 0.13±0.61 in the control group (21). Similar to our study, they found MN value higher in those exposed to chemicals in the printing press.

Şengün et al using the MN test in a study of the genotoxic and / or cytotoxic effects of a fluoride gel in human buccal epithelial cells, similar to our study, found that the use of acidulated phosphated fluoride (APF) gel increased the incidence of MN in oral buccal mucosa epithelial cells (22).

In a study examining the effect of Maraş grass use on the formation of MN by Nağaş S, the mean MN frequency was

found to be 7.27 in individuals using Maraş grass, while this value was found to be 4.30 in the control group (23).

In the study performed by Özçakmak et al. and evaluated the serum, muscle carnitine level and histopathological findings in rats treated with isotretinoin, it was observed that liver enzymes, serum carnitine levels could be increased and total protein levels could be decreased in rat model using high dose isotretinoin. He concluded that high-dose isotretinoin-induced serum and muscle carnitine levels may be reduced and neutrophilic myositis may occur, and that these side effects with carnitine supplementation can be prevented (16).

In a study by Yüksek J., isotretinoin investigated the effect of isotretinoin on some inflammation mediators (CD3 (+) T cell, CD4 (+) T cell, ICAM-1) in order to elucidate the anti-inflammatory mechanism of isotretinoin treatment in acne. The data obtained showed that perivascular and periadnexal CD3 (+) and CD4 (+) T cell levels were high in inflammatory lesions of acne cases and this level was significantly decreased with isotretinoin treatment. The decrease in CD3 (+) and CD4 (+) T cells, which are thought to play a role in acne inflammation after isotretinoin treatment, indicated that it is possible that the drug performs at least some of its anti-inflammatory activity (8).

Another conducted by Tütüncü D. on the effects of acne vulgaris treatment on nasal mucosa staphylococcal colonization, metisiline-sensitive Staphylococcus aureus (MSSA) growth was decreased in 20 patients who received systemic isotretinoin. In addition, methicillin-resistant Staphylococcus aureus (MRSA) growth was found to be slightly increased, while the total frequency of S. aureus colony decreased slightly but not significantly. In the study of Tütüncü D. the decrease in systemic isotretinoin and MSSA may be due to the decrease in the number of microorganisms carried from the skin around the nose to the nasal mucosa as a result of the decrease in papulopustular acne lesions (10).

In our study, when we examined the MN frequency between the experimental and control groups, the MN frequency in the experimental group was 109.4 ± 13.84, and 96.2 ± 10.13 in the control group. The difference between the experimental and control groups in terms of MN frequency was found to be significant and consistent with the data in the literature.

Acne vulgaris is a chronic, inflammatory disease of the pilosebaceous unit. It is mainly seen in adolescence (12). 4 main factors play a role in the pathogenesis; increased sebum production, ductal hypercornification, P. acnes colonization and inflammation. Systemic isotretinoin has been used in the treatment of acne since 1982. It is the only treatment agent effective for all four main factors involved in the pathogenesis of acne, but it has many side effects in addition to providing effective results in acne treatment (1,9,14).

There are numerous experimental studies in the literature to investigate the effects and side effects of isotretinoin. In our study, we examined the genotoxicity of isotretinoin by examining the incidence of MN formation and found a significant difference between the incidence of MN between isotretinoin and non-isotretinoin users. It is very important to determine whether the physical and chemical agents that have the potential to have genotoxic effects have mutagenic, carcinogenic and teratogenic effects. The reason is that the results from genetic toxicity tests show that many mutagenic substances are also carcinogenic.

CONCLUSION

In conclusion, we would like to emphasize that isotretinoin indication in acne treatment is very important for patients, relatives and public health. In addition, since MN formation is known to cause teratogenicity in pregnancy, it should be ensured that pregnancy is excluded before the use of isotretinoin, especially in women of childbearing age, and effective contraception measures should be taken during treatment and up to one month after the end of treatment. Patient should be informed correctly, risks of treatment should be explained and awareness should be raised.

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Ebru Derici Eker ORCID: 0000-0002-7094-7625
Serkar Kocoglu ORCID: 0000-0002-2061-1242
Nefise Ozlen Sahin ORCID: 0000-0003-2357-8262

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