

# The Effect of Oral Isotretinoin on Bone Healing in Rabbit Rhinoplasty Model: An Experimental Study

## Oral İsoetretinoinin Tavşan Rinoplasti Modelinde Kemik İyileşmesine Etkisi: DeneySEL Çalışma

Şahin Öğreden<sup>1</sup>, Hasan Deniz Tansuker<sup>1</sup>, Funda Emre<sup>2</sup>

<sup>1</sup>İstanbul Bağcılar Training And Research Hospital, Clinic of Otolaryngology, İstanbul, Turkey

<sup>2</sup>İstanbul Bağcılar Training And Research Hospital, Clinic of Pathology, İstanbul, Turkey

### ABSTRACT

**Introduction:** Since the age at which rhinoplasty is frequently performed is also the age at which acne is frequently seen, a person may be a candidate for both this surgery and isotretinoin therapy. In this study, we aimed to investigate the effect of oral isotretinoin on bone healing in rabbit rhinoplasty model.

**Methods:** Twelve one-year-old New Zealand white rabbits (3-4 g, all male) were included in the study. The animals were divided into two groups as experimental (n=6) and control group (n=6). One mg/kg oral isotretinoin and olive oil mixture was given each day orally to the experimental group starting from the surgery day. Lateral nasal osteotomy was performed starting from the distal end of the nasal bone. The rabbits were sacrificed 4 weeks later and the nasal bone specimens were sent for histopathological examination to evaluate bone healing between the groups. Bone healing was classified according to the grading described by Huddleston.

**Results:** Grade 1 bone healing was observed in four samples (66.7%) and grade 2 healing was observed in two samples (33.3%) in the study group. In the control group, three samples (50%) had grade 2 bone healing and the other three samples (50%) had grade 3 bone healing. The difference between the groups was statistically significant (p=0.027).

**Conclusion:** Bone healing was found to be slower in the isotretinoin group than in the untreated group. Although there are studies in the literature reporting that isotretinoin has a positive effect on bone healing, we observed opposite results. Therefore, we think that more experimental and clinical studies are needed to clarify this effect.

**Keywords:** Isotretinoin, lateral nasal osteotomy, bone healing

### ÖZ

**Amaç:** Burun estetiğinin sıklıkla yapıldığı yaş, aynı zamanda aknenin de sık görüldüğü yaş olduğundan, bir kişi hem bu ameliyat için hem de izotretinoin tedavisi için aday olabilir. Bu nedenle, çalışmamızda tavşan rinoplasti modelinde oral izotretinoinin kemik iyileşmesi üzerine etkisini araştırmayı amaçladık.

**Yöntemler:** On iki adet bir yaşında Yeni Zelanda beyaz tavşanı (3-4 g, tümü erkek) çalışmaya dahil edildi. Hayvanlar deneysel (n=6) ve kontrol grubu olmak üzere iki gruba ayrıldı. Operasyon gününden itibaren deney grubuna her gün oral olarak 1 mg/kg oral izotretinoin ve zeytinyağı karışımı verildi. Nazal kemiğin distal ucundan başlayan lateral nazal osteotomi yapıldı. Tavşanlar 4 hafta sonra sakrifiye edildi ve gruplar arasındaki kemik iyileşmesini değerlendirmek için nazal kemik örnekleri histopatolojik incelemeye gönderildi. Tavşanların kemik iyileşmesi, Huddleston tariflenen kemik iyileşme skoruna göre değerlendirildi.

**Bulgular:** Çalışma grubundaki 4 örnekte (%66,7) 1.derece kemik iyileşmesi ve 2 örnekte (%33,3) 2. derece kemik iyileşmesi gözlemlendi. Kontrol grubunda ise 3 örnekte (%50) 2. derece kemik iyileşmesi, diğer 3 örnekte (%50) 3.derece kemik iyileşmesi mevcuttu. Gruplar arasındaki fark istatistiksel olarak anlamlıydı (p=0.027).

**Sonuç:** İzotretinoin grubundaki kemik iyileşmesi, tedavi edilmemiş gruba göre daha yavaş bulundu. Literatürde izotretinoinin kemik iyileşmesi üzerinde olumlu etkisi olduğunu bildiren çalışmalar olmasına rağmen, biz çalışmamızda bunun karşıt sonuçlarını gözlemledik. Bu nedenle, bu etkiyi açıklığa kavuşturmak için daha fazla deneysel ve klinik çalışmaya ihtiyaç olduğunu düşünüyoruz.

**Anahtar Kelimeler:** İzotretinoin, lateral nazal osteotomi, kemik iyileşmesi



**Address for Correspondence/Yazışma Adresi:** Şahin Öğreden MD, İstanbul Bağcılar Training And Research Hospital, Clinic of Otolaryngology, İstanbul, Turkey

**Phone:** +90 505 808 14 20 **E-mail:** drsahinogreden@gmail.com **ORCID ID:** orcid.org/0000-0001-5513-8224

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## Introduction

Rhinoplasty is a very common aesthetic surgery that has become more popular day by day. Lateral osteotomy, which is one of the most challenging stages of this surgery, can cause damage to the mucosa and periosteum enveloping the bone tissue (1). It is important to ensure adequate mobilization and not to damage soft and supporting tissues when performing osteotomy. Excessive damage to these tissues will not only cause postoperative bleeding, desepithelization, prolonged ecchymosis and edema, but may also result in unwanted aesthetic and unpredicted functional outcomes (2).

Isotretinoin (13 cis retinoic acid) is a vitamin A derivative used as a drug in the treatment of severe acne that does not respond to antimicrobial therapy. The daily dose of cystic acne treatment is between 0.5 and 2 mg for six months (3). However, as in hypervitaminosis A, lip dryness, decreased bone density, increased risk of fractures, liver lesions, inhibition of bone growth, increased cholesterol and triglyceride, changes in liver enzymes and alkaline phosphatase may be seen as side effects (4).

When fracture happens, bone tissue has a very precious regeneration and healing ability. Beside some other important cells and mediators, osteoclasts and osteoblasts have very crucial roles in this complicated process by synthesizing and mineralizing the bone matrix. Isotretinoin, which is used extensively in dermatology, is known to adversely affect bone healing by interrupting osteoclasts and osteoblasts during the healing process (5).

Since the age at which rhinoplasty is frequently performed is also the age that acne is frequently seen, so a person can be a candidate for both this surgery and isotretinoin therapy at the same time.

In this study, by using rabbit model, we aimed to investigate the effects of isotretinoin on lateral osteotomy that is usually performed during rhinoplasty. As far as we know, this is the first experimental study on this issue.

## Methods

### Experimental Groups

This study was performed between May and June 2018 at Istanbul Bağcılar Training and Research Hospital Laboratory of Experimental Animals in Istanbul, Turkey. The experimental protocol was approved by the Istanbul Training and Research Hospital Local Ethics Committee of the Experimental Animals (decision no: 2018-09, date: 26.02.2018). The study was conducted in accordance with the principles of the European Community guidelines on the use of laboratory animals. A total of 12 adult, male New Zealand white rabbits weighing approximately 3-4 kg were maintained under ideal conditions of feeding and management in a room with cycles of 12 hours light and 12 hours dark, with constant temperature and relative humidity (60-70%). The rabbits were divided into two groups (n=6 each) designated as the experimental group as group 1 and the control group as group 2 in order to evaluate the effect of oral isotretinoin on bone healing in the early and late post-operative phases of osteotomy.

### Drug Administration

Isotretinoin (Roaccutane®, Roche) is an lipid-soluble drug. Therefore, each capsule (Roaccutane 10 mg) was divided in a dark room and 1 mg dosage of drug was diluted in 10 mL olive oil (0.1 mg/mL). Transfer of drug was made with a black, covered box to avoid light. The mixture was given to group 1 on the day of surgery and following every morning by oral gavage by 1 mL/kg dosage for four weeks until euthanasia. Group 2 was used as control group in which only oral olive oil was given.

### Surgical Procedure

The rabbits in all groups were anesthetized with xylazine hydrochloride 10 mg/kg (Rompun, Bayer Drugs, İstanbul, Turkey) and 50 mg/kg ketamine hydrochloride 59 mg/kg (Ketalar, Eczacıbaşı Drugs, İstanbul, Turkey). Nasal dorsal skins were draped with povidone-iodine solution and shaved. After infiltration with 1% lidocaine and 1:100.000 epinephrine mixture (Jetokaine®), a vertical midline nasal dorsum skin incision with 5 cm diameter was made through the periosteum. Continuous osteotomy was performed starting from the distal end of the nasal bone and extending to the nasal bone radius using a 4 mm osteotome without guide. At the end of the surgery, the incised skin was sutured and closed. All rabbits were euthanized after four weeks by intracardiac pentobarbital injection. The nasal bones of the rabbits were resected from the bilateral frontal process of the maxillary bone and separated from the nasal spindle of the frontal bone. The specimens were fixed with 10% buffered formaldehyde. Hematoxylin-eosin was used for staining. Bone healing was classified according to the grading described by Huddleston et al. (6) Fracture-healing phases in each specimen was quantified with use of a scale that assigns a grade based on the relative percentages of fibrosis, cartilage formation, woven and mature bone development in the callus (6). 4 µm sections were taken. Using a microscope (B×51 Japan), histological grading was performed. The grading was as follows: grade 1-fibrous tissue, grade 2-fibrous tissue with less cartilage formation, grade 3-fibrous tissue and cartilage are in the same amount, grade 4-cartilage tissue only, grade 5-predominantly cartilage and less amount of woven bone, grade 6-equal amount of cartilage and immature bone development, grade 7-more as immature bone and less cartilage, grade 8-totally immature bone, grade 9-predominantly immature bone and less amount of mature bone and grade 10-mature (lamella) bone.

### Statistical Analysis

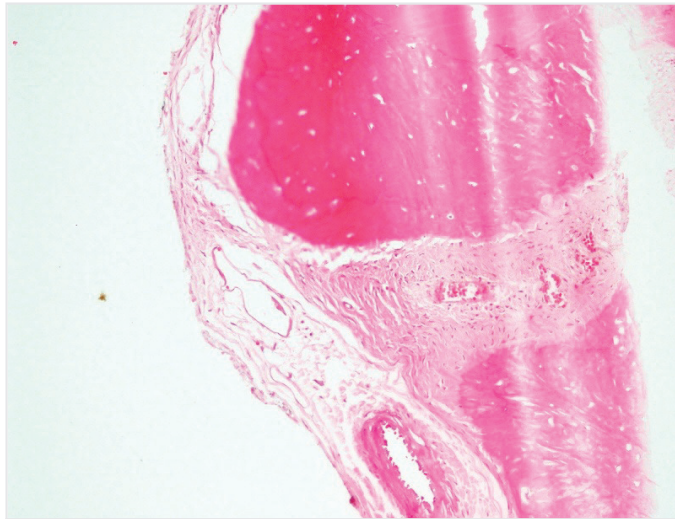
All analyses were conducted using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Correlations between categorical variables were assessed using the Pearson  $\chi^2$  test and chi-square test.  $P < 0.05$  was considered statistically significant.

## Results

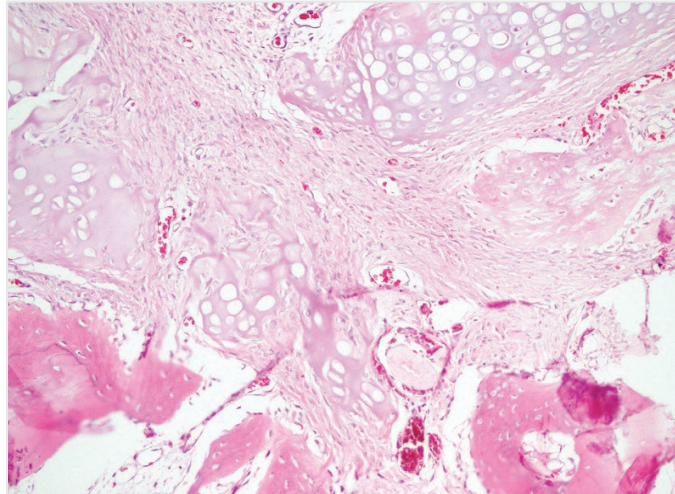
Grade 1 bone healing was observed in four samples (66.7%) and grade 2 bone healing was observed (Figure 1) in two samples (33.3%) in the study group. In the control group, three samples (50%) had grade 2 bone healing and the remaining three samples (50%) had grade 3 bone healing (Figure 2). The difference between the groups was statistically significant ( $p=0.027$ ) (Table 1).

**Table 1. Comparison of bone healing grades between the study (isotretinoin) and the control groups**

	Grade 1	Grade 2	Grade 3	p=0.027
Study group	4 (66.7%)	2 (33.3%)	-	
Control group	-	3 (50%)	3(50%)	
Total	4	5	3	



**Figure 1.** Grade 2 bone healing of the rabbit nasal bone; fibrous tissue is dominant with respect to cartilage tissue (hematoxylin-eosin, x100)



**Figure 2.** Grade 3 bone healing of the rabbit nasal bone; fibrous tissue formation is almost equal to cartilage tissue formation (hematoxylin-eosin, x100)

## Discussion

Kindmark et al. (7) reported that inhibition of the release of markers that provide bone turnover and calcium regulation at the beginning of isotretinoin treatment was inhibited and that this effect was reversible in the first 14 days despite the continuation of treatment (7). While the mechanism of action of isotretinoin is not fully understood, it is believed that this drug affects the cellular differentiation, growth, morphogenesis and apoptosis, controls tumor growth and modifies cellular cohesiveness. Isotretinoin reduces the size and activity of the sebaceous glands and produce a wound-healing-like pattern followed

by repair and remodeling of the skin (8,9). Nishio et al. (10) reported decreased inflammatory cells infiltrating large medullary areas, growth factors and fragmented thin bone formation in external retinoic acid given animals; which pointed out that isotretinoin had a negative effect on bone formation. Similar to this, we found that nasal bone healing was adversely affected in rabbits with isotretinoin treatment.

On the contrary, the rate of new bone formation in calvarial defect of the daily retinoic acid-treated group was reported to be higher than in the untreated group in a study performed on rats (11). Similar to this study, Kamm (12) reported that synthetic isotretinoin accelerated bone repair in their animal studies. Bergoli et al. (13) investigated the formation of new bone in the cavity formed after tooth extraction in a study conducted in 32 rats. In this study, new bone formation was reported to be faster in the isotretinoin-treated group on days 7,21,28 and 90. However, Valentic et al. (14) and Frankel et al. (15) reported higher rates of cortical and medullary bone resorption in long bones after giving high doses of vitamin A in rats. Similar to that, we observed grade 1 bone formation in four samples and grade 2 bone formation in two samples in the isotretinoin treated group in our study, which suggests that vitamin A might have a negative effect on bone healing.

The limiting feature of this study was that the number of rabbits was low and bone healing was not observed at different time periods.

## Conclusion

Although there are studies reporting that isotretinoin has a positive effect on bone healing in the literature, we observed opposite results. Therefore, we think that more experimental and clinical studies are needed to clarify this effect.

**Ethics Committee Approval:** The experimental protocol was approved by the İstanbul Training and Research Hospital Local Ethics Committee of the Experimental Animals (decision no: 2018-09, date: 26.02.2018).

**Informed Consent:** Informed consent was not obtained due to the study is an animal experiment.

**Peer-review:** Externally peer-reviewed.

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**Conflict of Interest:** No conflict of interest was declared by the authors.

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