Do non-steroidal anti-inflammatory agents prevent stenotic complications of tracheal surgery? The effects of tenoxicam on tracheal healing

Non-steroid antiinflamatuvar ajanlar trakea cerrahisinin striktürel komplikasyonlarını engeller mi? Tenoksikamın trakea iyileşmesi üzerine etkileri

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Background: Postoperative strictures, which are still serious problems of tracheal surgery, are caused by an inflammatory reaction with subsequent edema and granulation tissue formation. Taking this into consideration, we have designed a study in which tenoxicam (potent, non-steroidal, anti-inflammatory drug) was used after a tracheal surgery in rats.

Methods: Fourteen female Wistar rats were randomly divided into two groups (tenoxicam and control group) with equal numbers. Ketamine-xylazine anesthesia was performed in the rats. A vertical incision on the anterior tracheal wall, including the third to fifth cartilaginous rings, was performed and closed primarily with an interrupted suture technique using 4/0 absorbable suture. The tenoxicam group was administered 0.5 mg/kg/day tenoxicam and the control group was given 0.5 cc/day 0.9% NaCl via intraperitoneal route for 10 days beginning from the operation day. After two weeks, all animals were sacrificed under general anesthesia. Tracheas were excised, and a pathologist blindly evaluated the cases. The Mann-Whitney U-test was used for statistical analysis, and a value of $p<0.05$ was considered significant.

Results: The rat specimens were histologically evaluated and scored for inflammatory cell infiltration, angiogenesis, fibroblast proliferation, collagen deposition, and epithelial regeneration. There were meaningful differences in fibroblast proliferation ($p=0.036$) and epithelial regeneration ($p=0.002$). These results show that epithelial regeneration was higher and fibroblast proliferation was lower in the tenoxicam group.

Conclusion: Increased fibroblastic activity causes stenosis after tracheal surgery and the application of tenoxicam diminishes fibroblast proliferation and improves epithelial healing. Therefore, postoperative non-steroidal anti-inflammatory drug usage might be a useful therapy in the prevention of stenotic complications after tracheal surgery. However, further studies are needed.

Key words: Structure; tenoxicam; tracheal healing; tracheal surgery.

Amaç: Halen trakea cerrahisinin önemli komplikasyonlarından olan ameliyat sonrası striktürler, bir inflamatuvar reaksiyon ve devamında oluşan ödem ve granülasyon dokusu sonucu oluşur. Bunu göz önünde bulundurarak trakea cerrahisi uygulanan sıçanlarda tenoksikamın (potent bir non-steroid antiinflamatuvar ilaç olarak) kullanıldığı bir çalışma tasarladık.

Çalışma planı: On dört adet dişi Wistar sıçan rastgele şekilde, eşit sayıda iki gruba (tenoksikam ve kontrol grubu) ayrıldı. Sıçanlara ketamin-ksilazin anestezisi uygulandı. Anteriyor trakea duvarı üzerinde 3'den 5. kıkırdak halkasına kadar vertikal insizyon yapıldı ve 4/0 emilebilir sütür materyali kullanılarak kesilmiş dikiş tekniği ile primer olarak kapatıldı. Ameliyat gününden başlanarak 10 gün boyunca intraperitoneal yolla tenoksikam grubuna 0.5 mg/kg/gün tenoksikam ve kontrol grubuna 0.5 cc/gün %0.9'luk NaCl solüsyonu uygulandı. İki hafta sonra tüm hayvanlar genel anestezi altında sakrifiye edildi. Trakealar eksize edildi ve olgular yapılan uygulamalardan habersiz bir patolog tarafından incelendi. Mann-Whitney U-test ile istatistiksel analiz yapıldı ve $p<0.05$ değeri anlamlı kabul edildi.

Bulgular: Sıçanlara ait örnekler histolojik olarak değerlendirildi ve inflamatuvar hücre infiltrasyonu, anjiyogenez, fibroblast proliferasyonu, kollajen birikimi ve epitelyal rejenerasyon açısından skorlandı. Fibroblast proliferasyonu ($p=0.036$) ve epiteliyal rejenerasyon ($p=0.002$) konusunda anlamlı farklılıklar tespit edildi. Elde edilen bu sonuçlar tenoksikam grubunda epiteliyal rejenerasyonunun daha yüksek, fibroblast proliferasyonunun ise daha düşük olduğunu gösterdi.

Sonuç: Trakea ameliyatı sonrası artmış fibroblastik aktivite stenoza neden olur ve tenoksikam uygulaması fibroblast proliferasyonunu azaltır ve epiteliyal iyileşmeyi artırır. Dolayısıyla, ameliyat sonrası non-stroidal antiinflamatuvar ajan kullanımı trakea cerrahisinde striktürel komplikasyonları önlemek için faydalı bir yöntem olabilir. Ancak, ilave çalışmalara gereksinim vardır.

Anahtar sözcükler: Striktür; tenoksikam; trakea iyileşmesi; trakea cerrahisi.

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Impaired airway healing and strictures are still serious problems of tracheal surgery. Postoperative narrowing of the tracheal lumen is caused by an inflammatory reaction with subsequent edema and granulation tissue.[1] Although improved surgical techniques and suture materials have diminished the stenotic complications of tracheal surgery, we still have to find a solution to further diminish this complication rate. We think that the solution should be less surgical and more medical.

Considering the inflammatory cause of stricture, we thought that non-steroidal anti-inflammatory drugs (NSAIDs) may prevent or diminish strictures and stenosis after tracheal surgery. Therefore, we designed the current study in which tenoxicam (a potent NSAID) was used after tracheal surgery in rats.

MATERIALS AND METHODS

Fourteen female Wistar albino rats weighing 220-240 g were included in the study. They were randomly divided into two groups: the tenoxicam group (n=7) and the control group (n=7). The animals were anesthetized with 50 mg/kg ketamine hydrochloride (Alfamine, Alfasan Woerden, Holland) and 5 mg/kg xylazine hydrochloride (Alfazyne, Alfasan Woerden, Holland). The anesthetic agents were administered through the intraperitoneal route.

The animals were placed in a supine position. A vertical midline cervical incision was made, and strap muscles were retracted laterally. After exposure of the trachea, a vertical incision was made on the anterior tracheal wall including the third to fifth cartilaginous rings and closed primarily with an interrupted suture technique using 4/0 absorbable (polyglactine) sutures. Any intervention, such as intubation, was not needed for respiration since we did not obstruct the airway during the surgical procedure. After the surgical procedure, the animals were placed under a heating lamp until recovery from anesthesia. For postoperative care, 25 mg/kg/day cefazolin sodium for infection prophylaxis and 2 mg/kg/day tramadol HCL for analgesia were administered intraperitoneally to all animals.

The tenoxicam group had 0.5 mg/kg/day intraperitoneal tenoxicam (Oksamen, Mustafa Nevzat Ilaç Sanayi AŞ, İstanbul, Turkey), and the control group had 0.5 cc/day 0.9% NaCl for 10 days beginning from the day of the operation. After two weeks, all animals were sacrificed under general anesthesia. The trachea was excised from cricoid cartilage to carina with adjacent esophagus. The excised specimens were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax for histological examination. One pathologist blindly evaluated the cases after hematoxylin and eosin staining along with trichrome staining.

The Ehrlich/Hunt numeric scale[2] was used for the scoring of inflammatory cell infiltration, angiogenesis, fibroblast proliferation, and collagen deposition. The scores were 0 for absence, 1 for occasional presence, 2 for light scattering, 3 for abundance, and 4 for confluence of cells or fibers. Epithelial regeneration was scored according to Loewen's study.[3] The scores were 0 for no epithelium, 1 for single layer epithelium, and 2 for multilayer epithelium with complete closure for airway healing.

The statistical analysis for group comparison was made with the Mann-Whitney U-test, and the value of $p<0.05$ was considered as significant.

The current study was reviewed and approved by Ethics Committee for Animal Studies at Düzce University, Düzce-Turkey. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.[4]

RESULTS

None of the rats died during the surgical procedure or postoperative care. One rat had a simple skin infection which did not extend deep to the trachea. Other rats did not have any complications.

The macroscopic examination after excision of operated tracheas did not reveal any dehiscence of the suture line or obstruction of the lumen in the operated part.

The rat specimens were histologically evaluated and scored for inflammatory cell infiltration, angiogenesis, fibroblast proliferation, collagen deposition, and epithelial regeneration. Two rats from the tenoxicam group were excluded from the study during histopathological examination because of technical reasons. The scores are presented in table 1.

We found meaningful differences in fibroblast proliferation ($p=0.036$; <0.05) and epithelial regeneration ($p=0.002$; <0.05). Hence, epithelial regeneration was higher, and fibroblast proliferation was lower in the tenoxicam group (Figure 1, 2). However, the differences in inflammation, angiogenesis, and collagen deposition did not reach statistical significance.

DISCUSSION

The wound healing process starts in the suture line after the tracheal resection and anastomosis. The first step of wound healing is an inflammatory reaction in which epithelial, endothelial, inflammatory cells along with

Table 1. Histological scoring of the cases

Cases	Inflammation	Angiogenesis	Fibroblast proliferation	Collagen deposition	Epithelial regeneration
T1	1	1	1	0	2
T2	1	1	1	0	2
T3	1	1	1	0	2
T4	2	1	0	0	2
T5	2	1	0	0	2
C1	3	3	3	0	0
C2	3	3	3	0	0
C3	2	2	2	0	1
C4	2	1	1	0	1
C5	1	1	1	0	1
C6	1	1	1	0	0
C7	1	1	1	0	1

T: Tenoxicam group; C: Control group.

platelets and fibroblasts come together and interact to restore the injured tissue.[5] During this healing process, increased fibroblastic activity generates retractile fibrous tissue that causes stenosis and airway obstruction.[6]

Anastomotic complications, including granulation at the anastomotic line and stenosis after tracheal resection and reconstruction, lead to severe morbidity.[7] Wright et al.[7] experienced anastomotic complications in 9% of 901 tracheal resection patients. Several studies searched for a way to prevent structures after tracheal surgery. Talas et al.[1] explored the effect of corticosteroids and vitamin A in a rat study and found that corticosteroids were impairing the healing process while vitamin A was reversing this effect. Liman et al.[5] studied the effects of estradiol and progesterone and found that these sex hormones inhibit massive collagen deposition and fibroblast proliferation in tracheal healing. They concluded that they may prevent tracheal stenosis. Gorur et al.[8] used hyperbaric oxygen therapy after tracheal anastomosis in rats and found that hyperbaric oxygen improves tracheal healing and diminishes complication rates. Mitomycin was used topically to decrease tracheal scars but results of some recent studies suggest that topical mitomycin is not effective for avoiding tracheal stenosis.[6,9]

Tenoxicam is an NSAID from the oxicam group that also has strong anti-inflammatory, analgesic, and antipyretic activities. Minimal side effects, prolonged half-life, and elimination not influenced by liver and kidney diseases have popularized its use.[10] Our study investigated the effects of tenoxicam on a histological level, including inflammatory cell

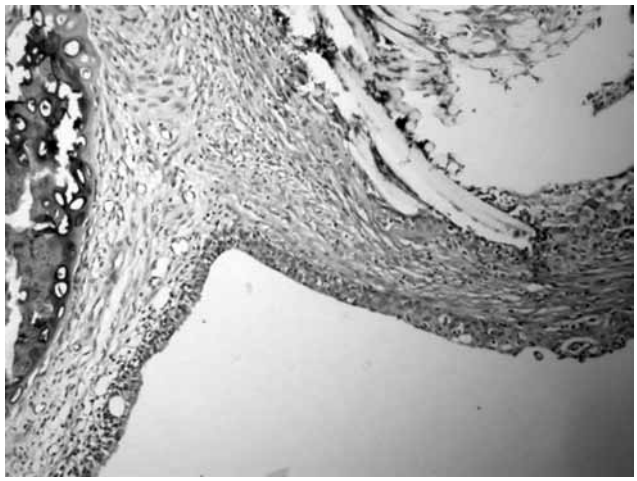

Figure 1. Photomicrograph of histopathology from tenoxicam group displaying less fibroblast proliferation and good epithelial regeneration (H-E x 200).

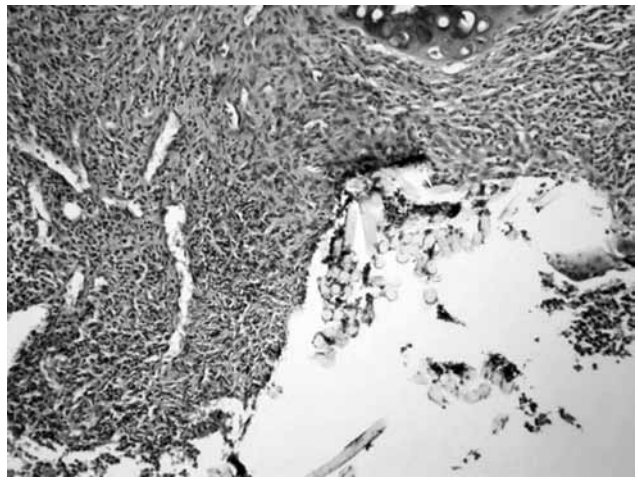

Figure 2. Photomicrograph of histopathology from control group displaying distinctive fibroblastic proliferation and impaired epithelial regeneration (H-E x 200).

infiltration, angiogenesis, fibroblast proliferation, and collagen deposition. Our statistical analysis displayed significant differences in fibroblast proliferation and epithelial regeneration. Fibroblast proliferation was less in the tenoxicam group than in the control group. Considering that increased fibroblastic activity is the cause of stenosis,[6] less fibroblast proliferation may be a sign of less stenotic complication. Epithelial regeneration was also better which, therefore, implies better wound healing in the tenoxicam group. We could not explain the pharmacological and histological basis of obtaining better epithelial regeneration when tenoxicam was used, but we think that obtaining the outcome of better wound healing in this study is important.

Our study included tenoxicam and control groups, but no sham-operated group. The lack of this group is one limitation of our study. Further studies, including a sham-operated group in which tenoxicam and other drugs are applied without any surgery performed, may yield stronger and more valuable results.

In conclusion, the application of postoperative NSAIDs may be a useful therapy in the prevention of stenotic complications of tracheal surgery. However, some further experimental and clinical studies are needed before their routine clinical usage.

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