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Experimental Study / *Deneysel Çalışma*

The Effect of Vitamin E-Coated Tracheotomy Cannula on Tracheal Reactive Oxygen Species

Vitamin E ile Kaplanmış Trakeotomi Kanülünün Trakeal Reaktif Oksijen Türleri Üzerine Etkisi

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Objectives: Iatrogenic airway injury after tracheotomy continues to be a serious clinical problem. Tracheotomy tubes can cause severe stomal stenosis in the trachea or infraglottic region. Vitamin E is a scavenger of different free radicals by working as an antioxidant. The aim of the present study was to evaluate the effect of vitamin E-coated tracheotomy tube insertion on the quantity of free radicals in rat tracheal tissue.

Materials and Methods: Male Sprague-Dawley rats were divided into three groups of six animals each. Ordinary tracheotomy tubes were applied to the first group and vitamin E-coated tracheotomy tubes were applied to the second group. The third group was used as control. Animals were killed and chemiluminescence measurements were made for tracheal tissue.

Results: Reactive oxygen species (ROS) levels were significantly increased in the first group of rats compared to those in control animals. ROS levels were statistically significantly decreased in the second group as compared to the first group.

Conclusion: Our results indicate that vitamin E decreases tracheotomy-induced ROS levels in tracheal tissue.

Key words: Tracheotomy; free oxygen radicals; vitamin E.

Amaç: Trakeotomi sonrası oluşan iyatrojenik havayolu travması önemli bir klinik sorun olmaya devam etmektedir. Trakeotomi kanülleri trakeada ve infraglottik bölgede önemli derecede darlık yapabilmektedir. Vitamin E bir antioksidan görevi görerek farklı serbest radikalleri ortadan kaldırır. Bu çalışmada vitamin E ile kaplanmış trakeotomi kanülü uygulamasının sıçanların trakeal dokularındaki serbest oksijen radikal miktarına etkisini inceledik.

Gereçler ve Yöntemler: Erkek Sprague-Dawley sıçanlar her grupta altı hayvan olacak şekilde üç gruba ayrıldı. Birinci gruba normal trakeotomi kanülü, ikinci gruba ise E vitamini kaplanmış trakeotomi kanülü takıldı. Üçüncü grup kontrol grubu yapıldı. Hayvanlar sakrifiye edildi ve trakeal dokulara kemilüminesans incelemesi yapıldı.

Bulgular: Reaktif oksijen türlerinin (ROT) seviyesi birinci grupta kontrol grubuna göre anlamlı derecede yükselmişti. İkinci gruptaki ROT seviyeleri ise birinci grupla karşılaştırıldığında istatistiksel olarak anlamlı derecede düşük bulundu.

Sonuç: Sonuçlarımız göstermiştir ki, vitamin E trakeal dokuda trakeotomi nedeniyle oluşan ROT seviyesini düşürmektedir.

Anahtar sözcükler: Trakeotomi; serbest oksijen radikalleri; vitamin E.

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Tracheotomy is an operative procedure that creates a surgical airway in the cervical trachea. The four basic indications for a tracheotomy are to bypass the upper airway obstruction, aid respiration over prolonged periods, assist with the clearance of lower respiratory tract secretions, and prevent aspiration of oral and gastric secretions.[1] Three types of complications are associated with tracheotomy: immediate, early, and late. Laryngotracheal stenosis (LTS) is one of the most important late postoperative complications.[2] Goldenberg et al.[3] reported that tracheal stenosis was the most common complication, occurring in 1.8% of tracheotomy patients. Taş et al.[4] analyzed their tracheotomy series and found LTS occurred as high as 3.2%. Note that specific patient populations, such as pediatric patients, are more susceptible to complications related to tracheotomy. Parrilla et al.[5] analyzed pediatric tracheotomy complications and found that the tracheal stenosis rate was 5.3%.

Damage to the perichondrium and cartilage has been found to be necessary for airway stenosis to develop. Fibroblasts involved in the healing of small ulcerations constrict the edges of the wounds, and fibrous tissue proliferates into the lumen.[1] Treatment to suppress the constricting phase, applied immediately after airway injuries, could prevent LTS.[6] Free radical release could conceivably be caused by tissue trauma during the tracheotomy procedure and may be related to tracheotomy complications such as LTS.

To prevent the development of LTS, pharmacological therapies must be used before the beginning of the healing process or at an early stage. Pata et al.[2] used carnitine at the beginning of a tracheotomy to prevent LTS by measuring the free radical quantity in the blood.

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols that are fat-soluble vitamins with antioxidant properties. Of these, α-tocopherol has been studied the most as it has the highest bioavailability, with the body preferentially absorbing and using this form. Moreover, α-tocopherol has been claimed to be the most important lipid-soluble antioxidant, purportedly protecting cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction.

In our previous studies, we demonstrated that vitamin E can decrease the levels of reactive oxygen species (ROS) in the larynx and lungs resulting from cigarette smoke, and ROS in the tympanic membrane due to myringotomy.[7,8] In the present study, we used vitamin E-coated tracheotomy tubes to modulate wound healing by decreasing the ROS levels of the trachea mucosa.

MATERIALS AND METHODS

This study was approved by the Animal Research and Care Committee of our university. Eighteen healthy adult male Sprague–Dawley rats weighing 250-500 g were used. All animals were obtained from and kept in the Institutional Animal Research Laboratory at our university in ordinary cages under standard conditions with free access to food and water.

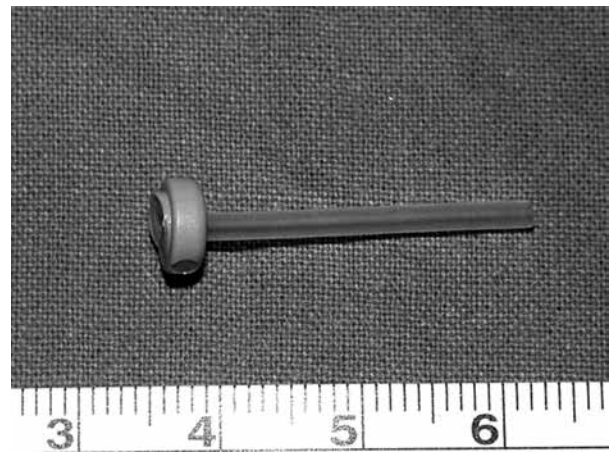

Fig. 1. *6F feeding tube modified for use as a tracheotomy tube in rats.*

For cannulation, a 6F feeding tube was used, which was cut into 25-mm lengths (Fig. 1). The vitamin E coating process was fabricated at the Department of Pharmaceutical Biotechnology Laboratory. Animals were randomly divided into three equal groups, and all animals were anesthetized with 50 mg/kg ketamine hydrochloride (Ketalar; Pfizer, New York, NY) by intraperitoneal injection. After removing the hair from the neck, the skin was cleaned with 10% polyvinylpyrrolidone iodine, and a vertical neck incision was made. The trachea was exposed, and vertical tracheotomy was carried out on the third and fourth tracheal rings. The 6F feeding tube was inserted as a tracheotomy tube, and a stoma suture with 3-0 silk was performed to fix the tube at the tracheotomy site (Fig. 2). Ordinary tracheotomy tubes were inserted in the first group, and vitamin E-coated tracheotomy tubes were inserted in the second group. The remaining group was used as control. In control group we exposed trachea for evaluation but we did not insert tracheotomy tube.

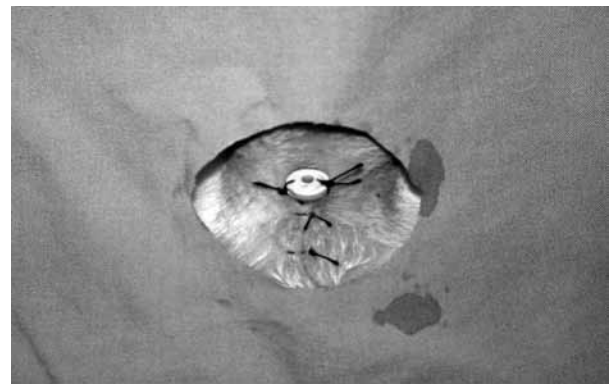

Fig. 2. *Tracheotomy tube inserted into a rat trachea.*

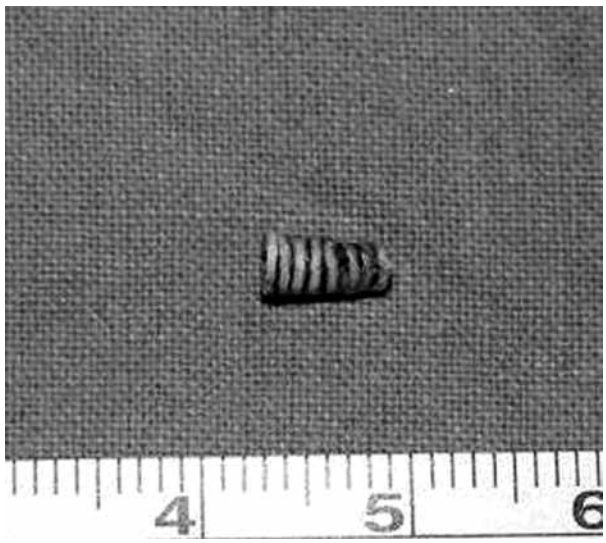

Fig. 3. *Resected tracheal tissue.*

All animals were decapitated 24 hours after tracheotomy, and tracheal tissues were removed. Tracheas were resected between the first and seventh rings for evaluation (Fig. 3). The tissues were washed with an ice-cold saline solution and processed immediately without storage to detect the ROS levels with chemiluminescence. Researcher who performed ROS measurements was unaware of the groups.

Detection of α-d-tocopherol

The α-d-tocopherol (potency 105,939 IU/g; Kocak Pharma, Istanbul, Turkey) was diluted with absolute alcohol, and peak values were determined spectrophotometrically (Biospec 1601; Shimadzu, Kyoto, Japan). A standard curve was drawn according to the known concentrations.

Preparation of α-tocopherol-absorbed tubes

An α-tocopherol solution was taken from the same stock and same concentration and was added to the tubes, which were then shaken at 20°C. The adsorption of α-tocopherol was spectrophotometrically determined, and the shaking process was continued until the maximum adsorption was reached. The α-tocopherol-absorbed tubes were sterilized at 120°C for 15 minutes, and the tubes were shaken with absolute ethanol and desorbed. The α-tocopherol concentration was measured spectrophotometrically at 292 nm, and the amount of α-tocopherol was calculated from a standard curve.

Table 1. Luminol–amplified chemiluminenscence values in rats tracheal mucosa (relative light unit per milligram tissue)

Group 1 (Standard tube)	Group 2 (Vitamin E-coated tube)	Group 3 (Control)
33.1	17.9	18.0
45.3	17.9	13.4
28.4	17.3	13.9
17.4	17.5	16.6
33.7	18.4	14.2
22.6	11.7	14.6

Chemiluminescence Measurement

The chemiluminescence measurements were made at room temperature using a Mini Lumat LB 9506 luminometer (EG&G Berthold, Bad Wilbad, Germany) in the presence of 0.2 mmol/L luminol. Counts were obtained at 5-second intervals, and the results were given as the area under curve (AUC) for a luminol chemiluminescence counting period of 5 minutes. The counts were corrected for wet tissue weight (rlu: relative light unit per milligram of tissue).

Statistical Analysis

InStat 3 (GraphPad Software, San Diego, CA, USA) was used for all analyses, and the results were reviewed by a biostatistician. Median differences of related parameters in the three groups were calculated using one-way analysis of variance (ANOVA). Bonferroni Comparisons Test was used for multiple comparisons of groups. The differences were considered significant when the P values were less than 0.05.

RESULTS

The luminol-amplified chemiluminescence levels in the tracheal tissues of all groups are shown in Table 1, and descriptive statistical data are summarized in Table 2. The mean luminol chemiluminescence level when ordinary tracheostomy tubes were inserted was found to be significantly greater (30.08±9.7 rlu/mg) compared to the control group (15.11±1.8 rlu/mg) (p=0.0041). The group with vitamin E-coated tracheostomy tubes was found to have a suppressed ROS level that was close to the level

Table 2. Descriptive statistical summary of tracheal mucosa ROS levels.

	Group 1 (n=6) (Standard tube)	Group 2 (n=6) (Vitamin E-coated tube)	Group 3 (n=6) (Control)
Median (range)	30.8 (17.4-45.3)	17.7 (11.7-18.4)	14.4 (13.4-18)
Mean±SD	30.1±9.7	16.8±2.5	15.1±1.8
SEM	3.97	1.02	0.73

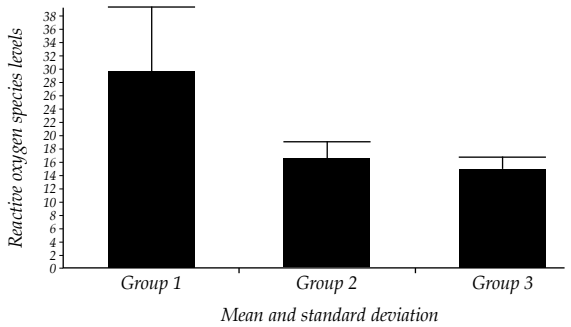

Fig. 4. *Chemiluminescence levels of groups (rlu/mg).*

of the control group (16.78±2.5 rlu/mg) as the values were not significantly different (p=0.2160) (Fig. 4).

DISCUSSION

The results of this study confirmed that tracheotomy tube insertion increases the quantity of ROS in the trachea and that coating the tube with vitamin E can decrease free radical production in traumatized rat trachea mucosa. To our knowledge, this is the first study to determine the effects of vitamin E-coated tracheotomy tube insertion on free radical levels in trachea mucosa.

Tracheotomy tubes cause mucosal compression when the pressure from the tube exceeds the capillary pressure of the mucosa. Mucosal edema, ischemia, and ulceration lead to perichondritis and chondritis. Healing occurs by secondary intention with granulation tissue proliferation and deposition of fibrous tissue, causing a weakened cartilage framework of the airway and narrowing of the lumen due to scarring.[9] LTS may result from external or surgical trauma through a similar mechanism as edema, granulation, and fibrosis.[10] Free radical release is conceivably caused by tissue trauma during the tracheotomy procedure and may be related to LTS.

Free radicals in the presence of oxygen may cause peroxidation of lipids within plasma and organellar membranes. Oxidative damage is initiated when the double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen-derived free radicals.[10] In a recent study, tissue malondialdehyde, a product of lipid peroxidation, was measured as an indicator of free radicals, and the authors reported that higher levels indicated higher concentrations of free radicals.[10] In an animal study, Pata et al.[2] determined that treatment with carnitine protected wounded tissue by measuring nitric oxide (NO) indirectly via nitrite and nitrate, malondialdehyde, and cholinesterase in the blood. Carnitine treatment partially prevented and significantly reduced the severity of tracheotomy-induced LTS.

Stenosis of the upper airway is a devastating and challenging disease for a surgeon to treat. The prevention of upper airway stenosis with pharmaceutical agents that modulate the wound healing process has been investigated.[11-13] Mitomycin C (MMC) has been widely applied for the prevention of stenosis, but MMC use for wound healing can cause major problems in the airway.[11] Halofuginone is a low-molecular-weight quinazolinone alkaloid coccidiostat that inhibits collagen type 1 synthesis, extracellular matrix deposition, and angiogenesis. It has been shown experimentally to be effective in preventing LTS caused by acute injury in dogs, but it must be administered through multiple routes.[12] Additionally, anti-transforming growth factor beta is a promising anti-scarring agent, but its use is limited by dosing, delivery techniques, and cost.[13] However, the literature does not contain any study that cover coating the tracheotomy tube with an antioxidant material. The tube coating procedure that we used did not require other pharmaceutical agents and is cost-effective. The application of vitamin E in this manner should be quite safe, and thus this coating procedure should be readily amenable for pediatric tracheotomies.

Reactive oxygen species have extremely brief half-lives. Consequently, free radical production is difficult to identify and quantity. The products of oxidative metabolism and the major mediators of free radical-induced damage are highest 24 hours after trauma,[14] so we measured ROS levels 24 hours after the tracheotomy. We used the chemiluminescence measurement method for the detection of ROS levels. Chemiluminescence, the emission of light during a chemical reaction, is a sensitive technique for estimating ROS generation that measures light produced as a by-product of oxidative metabolism.

In our study, chemiluminescence measurements demonstrated significant differences between the group with the vitamin E-coated tube and the group with the ordinary tube. Thus, our findings show that the vitamin E-coated tube reduced ROS production after trauma due to a tracheotomy tube. We are aware that the study groups are small, but the data are sufficiently convincing. The next step is to evaluate the histopathologic changes of the trachea mucosa under light microscopy after the insertion of a vitamin E-coated tube using large study groups.

Our results showed that trauma due to the insertion of a tracheotomy tube increases ROS levels and that coating a tracheotomy tube with vitamin E can decrease the ROS levels in the trachea. This study shows the short-term effect of vitamin E-coated tubes. Determining the long-term effects of these tubes on tracheal stenosis needs further pathological and clinical studies. In the future, the use of these antioxidant-coated tubes, especially for pediatric tracheotomies, may minimize tracheal stenosis, which can occur after a tracheotomy.

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