

# Effect of Epidermal Growth Factor on Rabbit Urethral Healing

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## Effect of Epidermal Growth Factor on Rabbit Urethral Healing

## Epidermal Growth Faktörün Tavşan Üretra İyileşmesindeki Rolü

### Summary

Extensive clinical and laboratory studies have demonstrated epidermal growth factor (EGF) to play an essential role in the regeneration and healing of epithelial surfaces, including respiratory, gastrointestinal and genitourinary tracts.

In this study, we evaluated the effects of supplemental EGF on urethral healing using a rabbit preparation.

Twenty New Zealand White rabbits were separated into three groups. In group 1 longitudinal urethrotomy and urethral repair were performed (n=8). In group 2 urethrotomy and urethral repair were performed as in group 1 and gelatin gel patches (1.2 x 0.5 x 0.1 cm) were placed between the repaired urethra and the skin (n=6). In group 3 gel patches containing 10 µg EGF were placed as in group 2 (n=6). Three weeks after surgery all rabbits were examined under anesthesia in order to determine macroscopic urethral fistulae, and the penis was harvested en bloc for histological examination.

There was evidence of macroscopic fistulae formation in only one animal in group 3. Histological evaluation revealed no statistically significant difference between the groups (p>0.05).

Despite literature suggesting beneficial effects of EGF on wound healing, we found no beneficial effects of EGF on urethral healing.

**Key Words:** Urethral healing, epidermal growth factor, wound healing, rabbits.

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### Özet

Epidermal growth faktörün (EGF) solunum, sindirim ve ürogenital sistem gibi epidermal yüzeyler içeren alanlar üzerindeki yenileyici ve yara iyileştirici özellikleri yapılan klinik ve deneysel çalışmalar ile gösterilmiştir.

Çalışmamızda, hazırladığımız tavşan modeli üzerinde EGF'nin üretra iyileşmesi üzerindeki olası etkilerini araştırdık.

Toplam 20 Yeni Zelanda tipi, beyaz, erişkin, erkek tavşan 3 gruba ayrıldı. Birinci gruba uzunlamasına üretra kesisi yapılarak onarıldı (n=8). İkinci gruba üretra kesisi ve üretral onarım ardından jelatin yapısında, 1.2x0.5x0.1 cm boyutlarındaki yama üretra ile cilt kesisi arasına yerleştirildi (n=6). Üçüncü gruba, 10 µg EGF içeren yamalar grup 2 de tarif edildiği gibi üretra onarımı sonrası tatbik edildi (n=6). Operasyonlardan 3 hafta sonra tavşanlar anestezi altında makroskopik fistül açısından değerlendirildikten sonra, bir bütün halinde çıkartılan penis dokuları histolojik olarak değerlendirildi.

Grup 3 de bir adet makroskopik fistül saptanırken, histolojik değerlendirmelere göre gruplar arasında anlamlı bir fark yoktu.

EGF'ün yara iyileşmesi üzerinde olumlu etkileri olduğunu belirten bir çok yayına rağmen, biz çalışmamızda üretra iyileşmesi üzerinde EGF'in olumlu etkisini gösteremedik.

**Anahtar Kelimeler :** Üretra iyileşmesi, epidermal growth faktör, yara iyileşmesi, tavşan.

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## INTRODUCTION

Normal wound healing consists of a cascade of cellular and biochemical events. The process is usually divided into three phases: the inflammatory phase, the proliferative phase and the remodeling phase<sup>1,2</sup>. Growth factors control the growth, differentiation and metabolism of cells during each of these three phases. Among the growth factors involved are transforming growth factor alpha (TGF- $\alpha$ ), transforming growth factor beta (TGF- $\beta$ ), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin-like growth factor (IGF)<sup>3</sup>.

EGF, a 6000 Da-molecular weight polypeptide composed of 53 amino acids, is released from platelets and keratinocytes, and stimulates mitogenic activity of keratinocytes, endothelial cells and fibroblasts<sup>3,4</sup>. The effects of EGF on target cells are mediated via the EGF receptor (EGFR) molecule on the plasma membrane<sup>5</sup>.

In human tissue, EGF is found in the skin and its appendages, the kidney, prostate and male genital tract. Large amounts are also excreted into human urine<sup>6-9</sup>. Similarly, the EGFR has been demonstrated in the human prostate, phallic skin, and rabbit prostatic urethra by both biochemical and immunohistochemical methods<sup>9-12</sup>.

Common complications of urethral surgery, such as fistula, diverticula and stricture, can be influenced by suture materials and/or urethral closing techniques<sup>13</sup>. Many techniques have been developed to decrease the risk of fistula, including meticulous tissue handling and suturing, avoidance of overlying suture lines, and the use of flaps of denuded epithelium, distal spongiosum or tunica vaginalis as a coverage layer<sup>14,15</sup>. However, none of these techniques are effective universally or available in each case. Furthermore, el-Galley et al. found deficiencies of EGF in the skin adjacent to hypospadias defects when compared to normal phallic skin. This may be related to the etiology of hypospadias and may help explain wound complication following surgery for hypospadias<sup>12</sup>.

EGF has been shown to play an essential role in the regeneration and healing of epithelial surfaces, including respiratory and corneal, and gastrointestinal and genitourinary tracts<sup>16-22</sup>. A study showing the positive effects of EGF in ureteroureteral anastomosis<sup>23</sup> and wound healing and conclusions reached about surgical complications in hypospadias cases that may be the result of low EGF levels led us to evaluate the effects of EGF in urethral healing.

Gelatin was chosen as the porous soft layer material since in practice it is more convenient than collagen and is known to have no antigenicity while collagen expresses some in physiological conditions. Gelatin is also far more economical than collagen<sup>24</sup>.

In this study, we evaluated the effects of supplemental EGF on urethral healing using a rabbit preparation.

## MATERIALS and METHODS

The protocol for the study was approved by the Ethical Committee of Abant İzzet Baysal University Medical School of Duzce.

**Materials:** EGF (mouse epidermal growth factor) was purchased from Serotec (England), gelatin was obtained from Teknik Ecza (Turkey), and glycerine was obtained from Merck (Germany).

**Preparation of gelatin-based gels:** 17% gelatin was dispersed in aqueous glycerol solution, and stirred on a waterbath at 40°C until homogenization. EGF was incorporated into gel formulation (pH=7.4), poured into molds and immediately refrigerated at 4°C until usage. Solidified gels were divided into 1.2 x 0.5 x 0.1 cm parts, each containing 10  $\mu$ g of EGF. Gels were exposed to UV for 1 h prior to in vivo applications to achieve sterilization<sup>24</sup>.

**Surgical procedure:** Adult New Zealand male rabbits ranging in weight from 2.5 kg to 3.2 kg were caged individually with free access to standard laboratory chow and tap water. The rabbits were anesthe-

tized with a mixture of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/ kg) IM.

Using sterile technique, the urethra was catheterized with an 8F feeding tube, the cavernous (pendulous) urethra was exposed through a ventral midline, penile skin incision (1.3 cm.) and mobilized from the corpora cavernosa. Longitudinal urethrotomy (1 cm) was performed. Urethra was then reclosed with running 7-0 polyglactin sutures and the skin with running 4-0 polyglactin sutures. The feeding tube was fixed to the meatus with 3-0 silk sutures and shortened so that only 1 cm was visible. Operations were performed with the aid of microsurgical loupes (2.5 X magnification).

The rabbits were then divided into three groups. In group 1, urethrotomy and urethral repair were performed as described (n=8). In group 2, urethrotomy and urethral repair were performed as in group 1 and gelatin gel patches (1.2x0.5x0.1 cm) were placed between repaired urethra and the skin (n=6). In group 3, gel patches containing EGF were placed as in group 2 (n=6).

The rabbits received enrofloxacin (Bayril, Bayer Corp.) intraoperatively (11.35 mg IV) and twice a day orally until catheter removal at day 7.

All animals were examined under anesthesia on day 21 in order to determine the presence of a macroscopic

urethral fistula using retrograde saline injection under pressure.

**Histological examination:** Animals were euthanized after testing using pentobarbital. The penis was harvested en bloc, and longitudinal sections were made through the area of interest. Specimens were fixed in 10% buffered formalin for at least 24 h. After dehydration in graded ethanol solutions, the specimens were embedded in paraffin, sectioned (4 µm), and stained with hematoxylin and eosin (H&E) and Gomori's trichrome. The sections were reviewed by a histologist unaware of the surgical technique used. Parameters assessed consisted of acute and chronic inflammation, fibrosis, squamous metaplasia, foreign body giant cells and subepithelial nests. Each parameter was scored from 0 (none evident) to 4+ (severe) (Table 1).

Groups were compared using Kruskal-Wallis and Mann-Whitney U tests. A p value of <0.05 was considered significant.

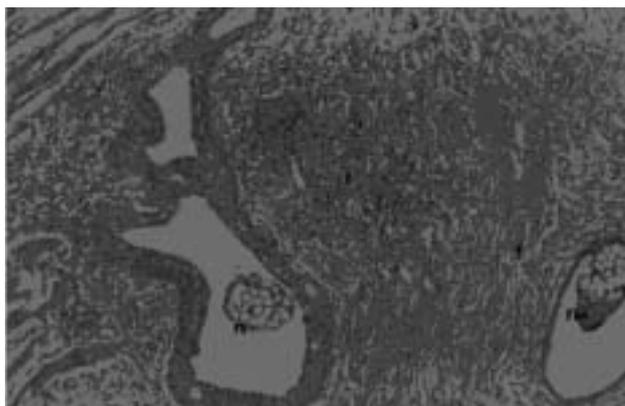
**RESULTS**

Rabbits tolerated the surgical procedure well and tolerated standard food by the morning of postoperative day 1. Animals had no problems with urination and wound dehiscence after the operation. There was evidence of macroscopic fistulae formation in only one animal in group 3.

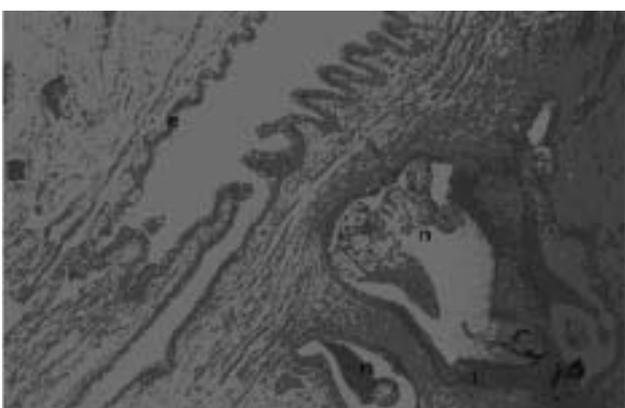
**Table 1.** Parameters assessed in the histological evaluation of urethral healing

	<b>Acute inflammation</b>	<b>Chronic inflammation</b>	<b>Fibrosis</b>	<b>Squamous metaplasia</b>	<b>Foreign body giant cells</b>	<b>Subepithelial nests</b>
	<i>(Increase of neutrophil number in subepithelial area)</i>	<i>(Increase of lymphocyte number in subepithelial area)</i>	<i>(Increase of collagen fibers in subepithelial area)</i>			<i>(Epithelial cells surrounding foci of persistent suture material)</i>
0	none	none	none	none	none	none
+	low	low	low	existent	existent	low
++	mild	mild	mild	-	-	mild
+++	moderate	moderate	moderate	-	-	moderate
++++	severe	severe	severe	-	-	severe

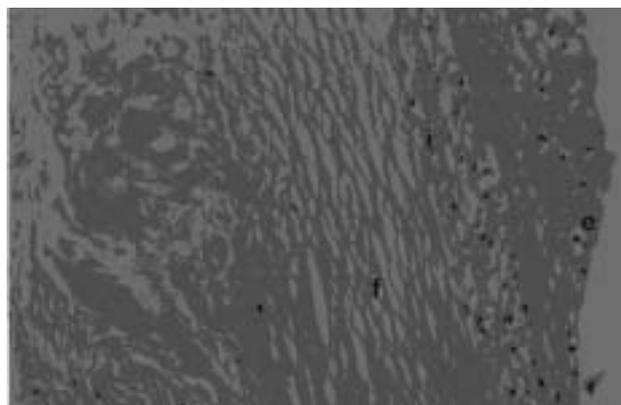
Rabbits in all groups had varying degrees of acute and chronic inflammation, foreign body giant cell formation, squamous metaplasia, fibrosis and subepithelial nests (Figs. 1, 2, 3). Histological evaluation revealed no statistically significant difference between the groups ( $p>0.05$ ) (Table 2).



**Figure 1.** Acute inflammation (i), subepithelial nest (n) and mild fibrosis (f) in urethra (H&E, X100).



**Figure 2.** Urethral epithelium (e) and subepithelial nest (n) (H&E, X40).



**Figure 3.** Urethral epithelium (e), acute inflammation (i) and fibrosis (f) (H&E, X400).

## DISCUSSION

We found that EGF supplementation with gels had no significant beneficial effect on urethral healing in this preparation. Control animals had no fistulae, and histological signs of inflammation were not prominent in any group.

We believe that our preparation was suboptimal for evaluating the effect of EGF supplementation. Because our control animals had no fistulae, it is clear that the EGF-supplemented group could not show better results. In other preparations, controls did not do as well<sup>25</sup>. Fistulae were observed, as well as subepithelial nests, inflammation, fibrosis, squamous metaplasia, and foreign body giant cells. Clearly a better preparation to evaluate the beneficial effects of EGF would require more severe injury, less care-

**Table 2.** Histologic evaluation of urethral healing

	Acute inflammation	Chronic inflammation	Fibrosis	Squamous metaplasia	Foreign body giant cells	Subepithelial nests
Group 1 (mean±SE)	2.125±0.40	1.75±0.45	2±0.45	0.125±0.13	0.125±0.13	2.625±0.38
Group 2 (mean±SE)	1.333±0.21	1.667±0.42	3±0	0±0	0.333±0.21	3±0.37
Group 3 (mean±SE)	2±0.52	1.5±0.22	2.333±0.42	0.333±0.21	0.5±0.34	2.167±0.17

ful suturing, shorter periods of catheterization, and different suture material, or a combination of the above. Furthermore, our preparation may not be a good model to investigate healing after repair of hypospadias. In the urethral wounds we created, EGF was normal, whereas in hypospadias, EGF may be insufficient.

We could not demonstrate any benefit of EGF supplementation in our preparation. This may be the result of excellent healing in controls or because EGF plays a rather menial role in urethral healing. This is unlikely, however, considering the beneficial effects seen in studies of EGF on healing of ureteroureteral anastomosis, wounds, corneal wounds, esophageal corrosive burns and gastric ulcers<sup>23, 26-30</sup>.

Another reason EGF was ineffective in our preparation could have been related to dosing. We used gelatin gels containing 10 µg EGF. This dosage lasts only a few hours in the wound. Perhaps a larger or longer-lasting dosage would have been more effective. On the other hand, excessive use of growth factors have the potential to lead to complications such as excessive scar formation and, theoretically, malignant transformation<sup>31</sup>.

Finally, we applied EGF in the wound. A better delivery system might be application directly on to the epithelium, i.e. the first layer of healing. This could be done using a urethral catheter that releases EGF directly on to the epithelial surface of the urethral wound, thereby benefiting epithelial regeneration at the wound surface without triggering epithelial regeneration in the deep layers of the wound leading to the formation of subepithelial nests and fistula formation.

In conclusion, despite literature suggesting beneficial effects of EGF on wound healing, we found no beneficial effects using our animal preparation. On the other hand, the evidence from previous studies would suggest that EGF supplementation may be beneficial if tested with other preparations and/or with other delivery systems.

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