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Original Article

The effect of epidermal growth factor applied locally for pelviureteral anastomoses

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Abstract: Objective: To investigate the effect of epidermal growth factor (EGF) on fibrous tissue formation and wound healing in experimental pelviureteral anastomosis (PUA). Materials and Methods: Twelve rabbits were divided equally into 2 groups: control (C) and epidermal growth factor (EGF). A 1-cm length of the ureteropelvic segment was resected through a laparotomy incision and then anastomosis was performed. The rabbits were administered locally with 100 μg/kg EGF (EGF group) all around the anastomosis lines after the surgical procedure. The C group did not receive any medication during their procedure. Intravenous pyelography was carried out on postoperative day 21. The rabbits were sacrificed and dissected under a dissecting microscope and examined for acute inflammation (AI), chronic inflammation (CI), granulation tissue amount (GTA), granulation tissue fibroblast maturation (GTFM), collagen deposition (CD), neovascularization (N), re-epithelization (R), and peripheral tissue reaction (PTR) in the anastomosis lines 3 weeks later. Results: There were no significant differences in the GTA, N, or R scores in the C group compared with those in the EGF group. In the EGF group, AI and CI scores were lower than they were in the C group. CD, GTFM, and PTR in the EGF group were higher than they were in the C group. Conclusion: EGF did not decrease CD, GTFM, or PTR, but did decrease important parameters in wound healing such as acute inflammation and chronic inflammation in an experimental model of PUA in rabbits.

Keywords: Epidermal growth factor, pelviureteral anastomoses, acute inflammation, chronic inflammation

Introduction

Pelviureteral repair is generally performed with interrupted absorbable suture material in a single layer. The four principles that Foley reported should be applied in open pyeloplasties: (a) the pelviureteral junction should be in funnel form after anastomosis, (b) the ureter should be anastomosed to the lowest point of the pelvis, (c) the anastomosis should be waterproof, and (d) there should be no tension in the anastomosis line. However, renal failure and hypertension, the morbidity and mortality of which are very high, can occur due to pelviureteral junction obstruction (PUJO), which is one of the postoperative complications [1]. Differential function of the kidney that underwent a surgical procedure cannot be corrected in 40% of cases because of contralateral stabilized hypertrophy in spite of successful pyeloplasty carried out in adults and children [2].

Ureteral catheter and double-J are used during primary anastomosis, and balloon dilatation and endopyelotomy in secondary obstructions to prevent these complications [1]. Endourological procedures are not appropriate in newborns and infants because of problems such as instrumentation difficulties, frequent radiation, and several anesthesia applications for stent settlement and extraction [1]. However, postoperative PUJOs remain despite all these surgical procedures. Epidermal growth factor (EGF) was used to increase fibrosis quality in several areas [3-6], but it has not been used simultaneously with primary anastomosis prophylactically to prevent PUJOs.

Although many treatment modalities have been suggested to prevent fibrosis, including Anderson-Hynes dismembered pyeloplasty, Foley Y-V pyeloplasty, and Culp-DeWeerd flap pyeloplasty [1], the optimal management protocol to treat fibrosis after pelviureteral anastomosis (PUA) remains controversial. Because none of these clinical approaches has gained popularity, research regarding pyeloplasty was conducted to reduce the risk of stricture formation [1].

When wound healing is considered, EGF is a rather useful healing agent in several areas such as healing of the esophagus, stomach, diabetic foot ulcers, and duodenal ulcers [7-10].

The aim of this study was to investigate the effect of EGF on fibrous tissue formation in experimental PUA.

Materials and methods

The experimental protocol was approved by the Ethical Committee of Selcuk University Faculty of Medicine (Konya, Turkey). Twelve 6-month-old male New Zealand rabbits weighing between 2500 and 3000 g were used. The rabbits were housed in cages with 1 animal per cage on sawdust bedding at a constant temperature of 21°C and humidity of 55% with 12-hour periods of light/dark exposure. The animals were allowed access to standard rabbit chow and water ad libitum. A 3-week period of acclimatization was used.

The rabbits were divided into two groups each containing 6 rabbits. The animals were fasted for 12 hours before the procedures. All surgical procedures were performed under ketamine (50 mg/kg, intramuscularly) and xylazine hydrochloride (5 mg/kg, intramuscularly) anesthesia by sterile technique. First of all, surgery was carried out through an abdominal midline incision to expose the ureter and to allow perioperative identification of the pelviureteral region. Second, 1-cm lengths of proximal ureteral segments and the renal pelvis were isolated from other tissues in both groups.

In the control group (C) a 1-cm length of the proximal ureteral segment was resected and then the anastomosis was performed using absorbable suture material [(6-0 DEXON); DAVIS + GECK, Inc., American Cyanamid Company, Manati, PR 00701, USA]. The laparotomy incision was then closed in standard fashion. The animals were not allowed to feed for the next 48 hours. The animals were fed parenterally for the first 2 days and later enterally.

After the same procedures were performed, 100 μg/kg EGF [SIGMA 100 μg/kg EGF, Chemical Company P.O. Box. 14508 St. Louis MO. 63108 USA] + 0.5 ml saline was administered locally all around the anastomosis lines in the EGF group.

The rabbits were fed orally on day 3 provided that there was no vomiting. Intravenous pyelography was carried out on postoperative day 21 to check whether there was pelviureteral leakage in the groups. There was no leakage in any of the study groups.

The rabbits were sacrificed after 3 weeks. A 1-cm segment of the ureter, which included both sides of the anastomosis in all groups, was removed to determine histopathologic findings.

The histopathologic evaluation was performed by a pathologist in a blind manner. Tissue specimens were fixed in 10% neutral buffered formaldehyde, processed routinely by an automatic tissue processor, and embedded in paraffin blocks. Sections of 4-μm thickness were stained with hematoxylin and eosin (H&E) for light microscopic morphologic evaluation. The extent of granulation tissue, granulation tissue fibroblast maturation, and collagen deposition were evaluated both immunohistochemically and light microscopically. FGFR1 (Abcam, Rabbit polyclonal FGFR1 (ab10646) and collagen type III (Abcam, Rabbit polyclonal to Col III. Ab7778) antibodies were used for immunohistochemistry [11-16]. The specimens were scored for differentiation of the healing and repair of the PUA. Histopathological parameters such as acute inflammation (presence of polymorphonuclear leucocytes at the anastomosis site), chronic inflammation (presence of lymphocytes and plasma cells), extent of granulation tissue (amount of granulation tissue), granulation tissue fibroblast maturation (maturation of granulation tissue; namely young, plump fibroblasts within a basophilic background or spindle fibroblasts compressed within a hyalinized background), collagen deposition (collagen matrix deposition between fibroblast bundles), re-epithelization (urothelial re-epithelization over the anastomosis site), neovascularization (amount of vascular proliferation during healing phase), and peripheral tissue reaction (amount of foreign body reaction and reactive changes in the surrounding tissues of the anastomosis site) were evaluated according to a 4-tiered system (score 0-3). We used a modified histologic scoring system developed specifically for this study based on the scoring system suggested in previous studies [11, 13]. According to the scoring system,

Table 1. Histopathologic evaluation of the pelviureteral junction in rabbits [median (min-max)]

Group	AI	CI	GTFM	CD	PTR	GTA	N	R
Control	1.0 (0-2)	1.5 (1-3)	1.0 (1-3)	1.5 (1-2)	1.0 (1-2)	2.0 (1-2)	1.0 (1-1)	3.0 (3-3)
EGF	0.0 (0-1)[x]	0.5 (0-1)[a]	3.0 (2-3)[b]	2.5 (1-3)	2.5 (2-3)[c]	1.0 (1-2)	1.0 (1-1)	3.0 (3-3)

EGF, Epidermal growth factor; AI, Acute inflammation; CI, Chronic inflammation; GTFM, Granulation tissue fibroblast maturation; CD, Collagen deposition; PTR, Peripheral tissue reaction; GTA, Granulation tissue amount; N, Neovascularization; R, Reepithelization. [a]P = 0.020 when compared with group Control, [b]P = 0.027 when compared with group Control, [c]P = 0.011 when compared with group Control, [x]P = 0.070 when compared with group Control (limited significance).

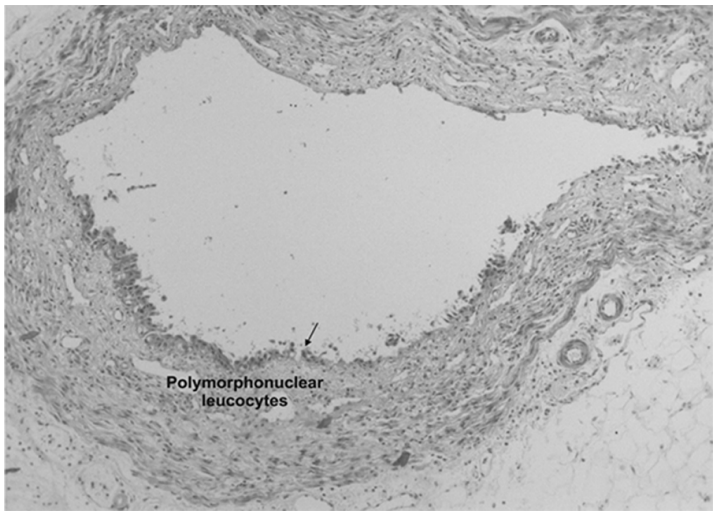

Figure 1. Photomicrograph of the C group showing the morphology of the acute inflammation (H&E).

the extent of polymorphonuclear leucocyte infiltration at the anastomosis site was graded as 0 if absent, and graded as 3 if extensive. All other morphologic parameters were evaluated according to the same principle. Histopathologic images were photographed by Zeiss Axio Imager A1 Microscope (Carl Zeiss Microimaging GmbH 37081 Göttingen, GERMANY) with a computerized digital camera system attached to it.

Data concerning acute inflammation, chronic inflammation, granulation tissue fibroblast maturation, collagen deposition, and peripheral tissue reaction were evaluated by Mann-Whitney U test. Comparisons for granulation tissue amount were analyzed by chi-square test because of values accumulated in two scores. Comparisons for neovascularization and re-epithelization were not performed because the same values were obtained in both groups. P values less than .05 were considered statistically significant for all tests. In all computations, SPSS version 20.0 (SPSS Inc, Chicago, IL, USA) was used.

Results

AI scores in the C [1.0(0-2)] group were significantly higher than those in the EGF [0.0(0-1)] group (p<.075) (**Table 1**). CI scores in the C [1.5(1-3)] group were significantly higher than those in the EGF [0.5(0-1)] group (p<.020) (**Table 1**).

GTFM scores in the EGF [3.0(2-3)] group were significantly higher than those in the C [1.0(1-3)] group (p<.027) (**Table 1**).

CD scores in the EGF [2.5(1-3)] group were significantly higher than those in the C [1.5(1-2)] group (p<.073) (**Table 1**).

PTR scores in the EGF [2.5(2-3)] group were significantly higher than those in the C [1.0(1-2)] group (p<.011) (**Table 1**).

There were no significant differences in the GTA scores in the C [2.0(1-2)] group compared with those in the EGF [1.0(1-2)] group (**Table 1**).

There were no significant differences in the neovascularization (N) scores in the C [1.0(1-1)] group compared with those in the EGF [1.0(1-1)] group (**Table 1**).

There were no significant differences in the re-epithelization (R) scores in the C [3.0(3-3)] group compared with those in the EGF [3.0(3-3)] group (**Table 1**).

EGF did not decrease collagen deposition, granulation tissue fibroblast maturation, or peripheral tissue reaction, but did decrease important parameters in wound healing such as acute inflammation (p<.075) (**Table 1**, **Figure 1**) and chronic inflammation (p<.020) (**Table 1**, **Figure 2**). EGF increased re-epithelization and decreased acute and chronic inflammation

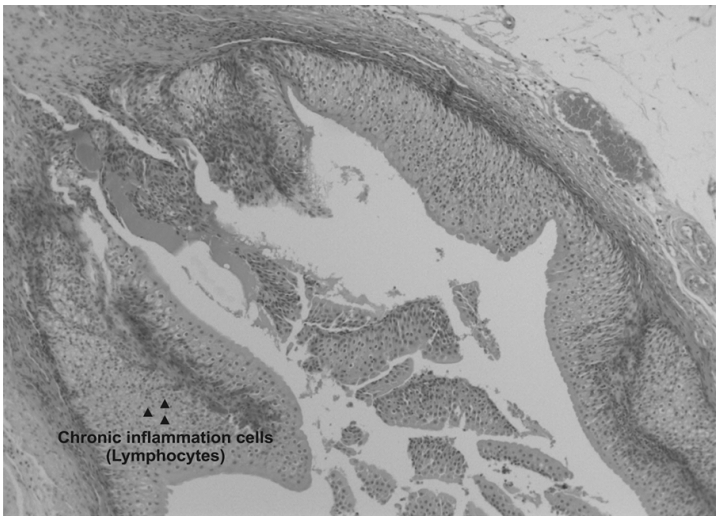

Figure 2. Photomicrograph of the C group showing the morphology of the chronic inflammation (H&E).

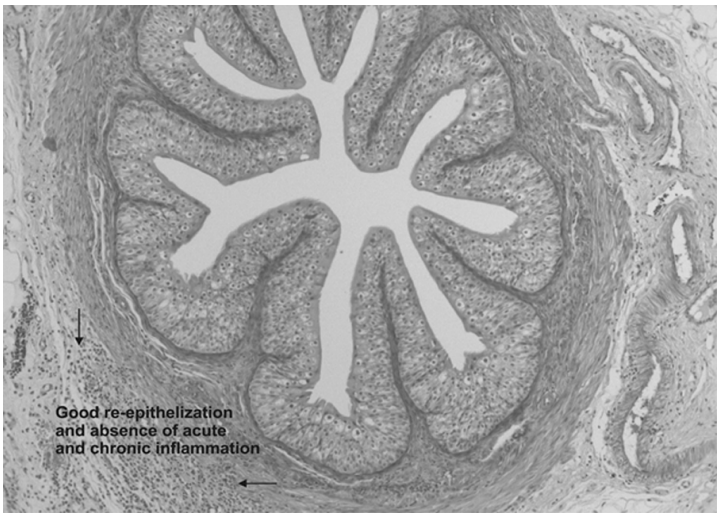

Figure 3. Photomicrograph of the EGF group showing the morphology of the good re-epithelization, and absence of acute and chronic inflammation (H&E).

(**Table 1**, **Figure 3**) in an experimental model of PUA in rabbits.

Discussion

In this pyeloplasty model, we used EGF as a wound healing agent, which had not been tried before in such a model. We demonstrated that EGF administration did not decrease granulation tissue amount, neovascularization, and re-epithelization, but did decrease important parameters in wound healing such as acute inflammation and chronic inflammation in an experimental model of PUA in rabbits. It is of interest that on postoperative day 21 PUA sections from EGF-treated rabbits appeared more normal than those of untreated rabbits and this confirmed the protective effect of EGF.

Early postoperative complications such as leakage and stricture seen after PUA are a major clinical problem with a high degree of morbidity. Most clinicians still use surgical procedures such as Anderson-Hynes dismembered pyeloplasty, Foley Y-V pyeloplasty, and Culp-DeWeerd flap pyeloplasty, facing the problem of recurrent ureteropelvic stricture after surgery. However, these various surgical procedures have been performed to prevent postoperative PUJOs, but it remains an important problem.

Epidermal growth factor is a mitogenic polypeptide. It starts to affect wound healing at the end of the inflammation phase, and it induces fibroblast process. Moreover, epidermal growth factor can stimulate the granulation tissue process and epithelization. In addition, it accelerates wound healing [17, 18]. EGF helps wound healing through ceratinocyte migration, fibroblast function, and granulation tissue formation [19-24]. However, EGF has not been used simultaneously with primary anastomosis prophylactically to prevent postoperative PUJOs.

The healing rate and degree of scar tissue formation were dependent on the concentration and number of applications of epidermal growth factor. Serial applications of concentrated epidermal growth factor accelerated healing and decreased scar tissue formation [7-10]. An agent such as EGF, which has been shown to reduce connective tissue overgrowth in healing wounds, might be expected to alter the pyeloplasty healing process in the rabbit model and thereby provide additional insight into the pathogenesis of PUJOs. AI, CI, GTA, GTFM, CD, re-epithelization (R), neovascularization (N),

and PTR in anastomosis lines could be considered reliable and objective anastomoses markers in scar tissue formation [8, 10].

A prior communication from our unit demonstrated for the first time that natrium hyaluronate did not decrease fibrosis, but increased important parameters in wound healing such as neovascularization and re-epithelization in an experimental model of pelviureteral anastomosis in rabbits [25]. Although this new information gave us a greater insight into the treatment of PUJO, many questions remained.

Our results showed that the local application of EGF significantly decreased AI and CI scores in the EGF group when compared with the C group. There were no significant differences, however, in terms of GTA, N, or R values in either group in an experimental model of PUA in rabbits.

In conclusion, our results indicate that EGF facilitates better PUA lines. EGF can be used simultaneously with pyeloplasty prophylactically to prevent postoperative PUJOs in children when acute inflammation and chronic inflammation are considered. We think that decreased acute inflammation and chronic inflammation in the EGF group are positive in terms of fibrous tissue formation. Further experimental and clinical studies are needed for detailed evaluation of EGF in PUA.

Disclosure of conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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