Investigation of Histopathologic Changes in Pelviureteral Anastomoses after Local Application of Fibrinogen and Thrombin

Muslim Yurtçu*, Sıdıka Findik†, Muhammet Emin Yurtçu‡ and Zeliha Füsun Baba§

1Department of Pediatric Surgery, Meram Medical School of Necmettin Erbakan University, Konya-TURKEY
2Department of Pathology, Meram Medical School of Necmettin Erbakan University, Istanbul-TURKEY
3Faculty of Pharmacy, Bezmiælem Vakif University, Istanbul-TURKEY
4Department of Pathology, Medical School of Acibadem University, Istanbul-TURKEY

*Corresponding author: Muslim Yurtçu, Department of Pediatric Surgery, Meram Medical School of Necmettin Erbakan University, Konya-TURKEY, Fax: +90 332 223 6181, Email: myurtcu@konya.edu.tr

Abstract

Introduction/background: Fibrinogen and thrombin can be considered as an alternative measure to improve the success of pyeloplasty.

Objective: This study aimed to evaluate the effect of FT on fibrous tissue formation in an experimental pelviureteral anastomosis (PUA).

Study design: Eighteen rabbits were included in the study. Rabbits were allocated in three groups (n:6): control (CG), sham (SG), and FT (FTG). In CG, a 1-cm-length of the ureteropelvic segment was resected. In SG, 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 220 miligrams fibrinogen and 1000 IU human thrombin combination (FTG) to the all-round of anastomosis lines after the surgical procedure. The SG group did not receive any medication. Intravenous pyelography was carried out on postoperative day 21. The rabbits were sacrificed and dissected and examined for acute inflammation (AI), chronic inflammation (CI), granulation tissue amount (GTA), granulation tissue fibroblast maturation (GTFM), collagen deposition (CD), neovascularization (N), reepithelialization (R), and peripheral tissue reaction (PTR) in the anastomosis lines 3 weeks later.

Results: Use of FT after pelviureteral anastomosis increases granulation tissue fibroblast maturation and collagen deposition expressions in pelviureteral tissue.

Discussion: This study focuses on the histopathological parameters such as AI, CI, GTA, GTFM, CD, reepithelialization, neovascularization, and PTR of experimental PUA.

Conclusion: Use of FT after PUA increases GTFM and CD expressions in pelviureteral tissue. FT can be considered as an alternative measure to improve the success of pyeloplasty and to prevent secondary pelviureteral junction obstruction.

Introduction

The success rate of pyeloplasties can be improved by interrupted absorbable suture material in a single layer, funnel form after anastomosis, ureter anastomosis to the lowest point of the pelvis, interposing a waterproof anastomosis between pelvis and ureter, and no tension in the anastomosis line. Despite advances in pyeloplasties and use of the above maneuvers, renal failure and hypertension, the morbidity and mortality due to Pelviureteral Junction Obstruction (PUJO) still remains a common complication [1].

Ureteral catheter and double-J are used during the primary anastomosis, balloon dilatation, and endopelotomy in secondary obstructions to prevent these complications [1]. Endourological procedures are not appropriate in new-borns and infants because there are some problems such as instrumentation difficulties, frequent radiation, and several anesthesia applications for stent settlement and extraction [1]. However, postoperative PUJO remains despite all these surgical procedures. Fibrinogen And Thrombin (FT) were used to increase the fibrosis quality in several areas [2-6], but it was not used simultaneously with primary anastomosis prophylactically to improve the success of pyeloplasty and to prevent anastomosis leakage.

Although many treatment modalities are suggested to prevent fibrosis, including Anderson-Hynes dismembered pyeloplasty, Foley Y-V pyeloplasty, and Culp-De Weerd
flap pyeoplasty [1], the optimal management protocol to treat the fibrosis after PUA remains controversial. When the success of pyeoplasty and preventing of anastomosis leakage are considered, FT is a rather useful healing agent in several areas such as healing of Urethrocutaneous Fistulas Repair (UCFR) [2] and preventing of UCFR after recurrent fistula repair [3].

The aim of the study was to investigate the effect of FT to improve the success of pyeoplasty and to prevent anastomosis leakage in experimental PUA.

**Material and Methods**

The experimental protocol was approved by the Ethical Committee of Necmettin Erbakan University Faculty of Medicine (Konya, Turkey). Eighteen 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 three 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 periopeative identification of the pelviureteral region. Secondly, 1 cm of proximal ureteral segments and renal pelvis were isolated from other tissues in three groups.

In the CG, a 1-cm-length of the ureteropelvic segment was resected through a laparotomy incision. In the SG, a 1-cm-length of the ureteropelvic segment was resected through a laparotomy incision, and then anastomosis was performed with absorbable suture material [(6 - 0 DEXON); DAVIS + GECK, Inc., American Cyanamid Company, Manati, PR 00701, USA] under a microscope. The SG group did not receive any medication during the procedure. In the FGT, after performing the same procedures, combination of 220 milligram’s fibrinogen and 1000 IU human thrombin was administered locally to the all-round of the anastomosis lines. The laparotomy incision was then closed in standard fashion in three groups. The animals were not allowed to feed for the next 48 hours. The animals were fed parenterally for the first 2 days and later enteral. All medical applications were performed under ketamine (50 mg/kg, intramuscularly) anesthesia by sterile technique. Intravenous pyelography was carried out on postoperative day 21 to check whether there was pelviureteral leakage in the groups. The rabbits were fed orally on day 3 on the condition that there was no vomiting. There was no leakage in any of the study groups. The rabbits were sacrificed after 3 weeks. A 1-cm pelviureteral segment, including both sides of the anastomosis in all groups, was taken (removed) to determine histopathologic findings.

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 masson trichome and light microscopically. The specimens were scored for the differentiation of healing and repairing 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 hyaline background), collagen deposition (collagen matrix deposition between fibroblast bundles), reepithelialization (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 [7-9]. According to the scoring system, the extent of polymorphonuclear leucocyte infiltration at the anastomosis site was graded as 0 if none and 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 Micro imaging GmbH 37081 Göttingen, GERMANY) with a computerized digital camera system attached to it.

The study was approved by the Local Ethical Committee of Necmettin Erbakan University Scientific Research Council (2010-041/31.05.2010).

Data concerning acute inflammation, chronic inflammation, granulation tissue amount, granulation tissue fibroblast maturation, collagen deposition, neovascularization, reepithelialization, and peripheral tissue reaction were evaluated by Kruskal-Wallis test. Comparisons for granulation tissue amount, granulation tissue fibroblast maturation, collagen deposition, and peripheral tissue reaction were analyzed by Kruskal-Wallis variance analysis, followed by Bonferroni adjusted Mann-Whitney U test because of values accumulated in two scores. Comparisons for acute inflammation, chronic inflammation, neovascularization, and reepithelialization were not evaluated because the three groups had the same values. P values less than .05 were considered statistically significant for all tests. In all computations, SPSS version 17.0 (SPSS Inc, Chicago, IL) was used.

**Results**

The values for AI, CI, GTA, GTFM, CD, N, R, and PTR of the pelviureteral anastomosis lines in groups were listed in Table 1 and 2. In the FTG group, GTA scores [1.330 ± 0.516] were significantly lower than the ones in the CG [2.170 ± 0.408] and SG [1.670 ± 0.516] (p = .041). In the FTG, GTFM scores [2.670 ± 0.516] were significantly higher the ones in the CG [1.330 ± 0.516] and SG [1.500 ± 0.837] (p=0.14). In the FTG, CD scores [2.170 ± 0.753] were significantly higher the ones in the CG [1.170 ± 0.408] and SG [1.500 ± 0.548] (p = .047). In the FTG, PTR scores [1.330 ± 0.516] were significantly lower than the ones in the CG [2.500 ± 0.548] (p = .010) (Table 1).
There were no significant differences in AI scores in the CG [1.330 ± 0.516] compared with those in the SG [1.000 ± 0.632] and FTG [0.500 ± 0.830] (p = .124) (Table 2).

There were no significant differences in CI scores in the CG [1.670 ± 0.516] compared with those in the SG [1.670 ± 0.816] and FTG [1.170 ± 0.408] (p=.246) (Table 2).

There were no significant differences in N scores in the CG [1.500 ± 0.548] compared with those in the SG [1.000 ± 0.000] and FTG [1.000 ± 0.000] (p =.053) (Table 2).

There were no significant differences in R scores in the CG [2.330 ± 1.033] compared with those in the SG [3.000 ± 0.000] and FTG [3.000 ± 0.000] (p=.119) (Table 2).

FT decreased granulation tissue amount in the FTG compared to the CG and the SG, but increased collagen deposition and granulation tissue fibroblast maturation in an experimental model of PUA in rabbits (Figures 1, 2, 3, and 4).

Discussion

Despite the advancements in pyeloplasty surgery, pelviureteral junction obstructions remain the most common complication. Interrupted absorbable suture material, funnel form and waterproof anastomosis, Anderson-Hynes dismembered pyeloplasty, Foley Y-V pyeloplasty, and Culp-De Weerd flap pyeloplasty were used to interpose a waterproof layer between renal pelvis and ureter to prevent obstruction formation [1]. The compromised blood supply of adjacent tissues
and scar formation after multiple repairs limit the use of tissue flaps and require other supporting materials [2]. Therefore, to prevent anastomosis leakage and obstruction, autologous or homologous fibrin materials and manufactured fibrin sealants are widely used [2]. In this pyeloplasty model, we used FT as a wound healing agent, which had not been tried before in such a model. We demonstrated that FT administration did not decrease granulation tissue amount and peripheral tissue reaction, but increased important parameters in wound healing, such as the wound healing rate, proliferation, and decreased collagen deposition in an experimental model of PUA in rabbits. It is of interest that at postoperative day 21 PUA sections from FT-treated rabbits appear more normal than those of untreated ones, and this confirmed the protective effect of FT.

Fibrinogen and thrombin combination is an agent which accelerates the wound healing and helps suture support via epithelisation at the end of inflammation phase. Also, it induces a tight covering with the fibrinogen/thrombin-based collagen fleeces [10,11]. However, FT was used successfully with primary anastomosis prophylactically to prevent postoperative PUJO.

The healing rate and degree of scar tissue formation depended on the concentration and number of applications of fibrinogen and thrombin. Serial applications of concentrated fibrinogen and thrombin shorten some measurements of coagulation initiation speed and produce a short-lived increase in endogenous thrombin potential, through increased clotting factors availability [12]. The availability of FT, known as an antiproliferative effect, might be expected to alter the pyeoplopy healing process in the rabbit model and thereby provide additional insight into the pathogenesis of PUJO [13].

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 reepithelization in an experimental model of pelviureteral anastomosis in rabbits [14]. Also, other prior communication from our unit demonstrated, for the first time, that epidermal growth factor 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 and chronic inflammation in an experimental model of PUA in rabbits [15]. Although this new information gave us a greater insight into the treatment of pelviureteral junction obstruction, many questions remained.

Our results showed that the local application of FT significantly decreased GTA and PTR scores in the FT group compared with the C group. But there were no significant differences with regard to AI, CI, N, and R values in three groups in experimental model of PUA in rabbits. We would like to demonstrate the results of immunochemistry and Western blot analyses with specific antibodies, as well as real-time PCR and so on. It was not possible because of deficiency of tissue and technical conditions.

Conclusions

Our results indicate that FT facilitates better PUA lines. FT can be used simultaneously with pyeoplopythelisation prophylactically to prevent postoperative PUJO in children when granulation tissue fibroblast maturation and collagen deposition are considered. We think that decreased granulation tissue amount and peripheral tissue reaction in the FTG are positive in terms of fibrous tissue formation. Further experimental and clinical studies are needed for detailed evaluation of FTG in PUA.

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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.

References