IS THERE A DIFFERENCE IN PLATELET-RICH PLASMA APPLICATION METHOD AND FREQUENCY TO PROTECT AGAINST URETHRA STRICTURE?

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ABSTRACT

Objective : To determine the efficacy of instillation frequency and submucosal injection of platelet-rich plasma (PRP) after urethral trauma to prevent urethral inflammation and spongiofibrosis.

Material-Method: Fifty rats were randomized into 5 groups, with 10 rats in each group. The urethras of all rats were traumatized with a pediatric urethrotome knife at 6 and 12 o'clock, except in the sham group. Group 1 was the sham group and had only urethral catheterization daily for 15 days, Group 2 was given 0.9% saline (physiologic saline [(UI+PS]) once a day after urethral injury (UI+ PS), Group 3 was injected with PRP submucosally after urethral injury, Group 4 was given PRP once a day as intraurethral instillation using a 22 Ga catheter sheath with urethral injury, and Group 5 was given PRP twice a day as intraurethral instillation using a 22 Ga catheter sheath with urethral injury.

Each administration of PRP was administered as 300 million platelets/150 microliters. On day 15, the penises of the rats were degloved to perform penectomy. Histopathologic evaluation was made for spongiofibrosis, inflammation, and congestion in vascular structures.

Results: When the sham group, UI+PS, UI+PRPx1, UI+PRPx2 and UI+PRPs groups are compared in total, there were significant differences identified for parameters other than edema. When the UI+PS, UI+PRPx1, UI+PRPx2 and UI+PRPs groups are compared, the UI+PS group was observed to have significantly more inflammation (mucosal inf. 2.42 ±0.53) and spongiofibrosis (2.42 ±0.53). All the PRP groups were identified to have significantly less mucosal inflammation (UI+PRPs 1±0, UI+PRPx1; 1.4±0.51, PRPx2; 1.33±0.5) and spongiofibrosis (UI+PRPs; 1.57±0.53, PRPx1; 1.2±0.42, PRPx2; 1.55±0.52). The group with lowest spongiofibrosis was seen to be the PRPx1 group.

Conclusion: This study showed that PRP significantly reduced mucosal inflammation and spongiofibrosis, independent of administration route, when applied to the urethra after urethral trauma.

KEYWORDS: Urethral Stricture, PRP, Urethral Fibrosis, Urethral inflammation, Urethral Healing

Introduction:

Urethra stricture forms due to narrowing of the lumen in any region of the urethra linked to fibrosis developing due to trauma, infection or idiopathic causes.[(1)] Prevalence appears to be 0.9% in males, and it is a disease with very high treatment costs.[(2)] Currently the most common cause of urethra stricture etiology is

iatrogenic interventions as a result of increasing endoscopic interventions, with most common development after TUR-P. ([3,4] Urethra stricture is a disease affecting all age groups and quality of life of the patient, with treatment using minimally invasive treatments and high recurrence rates. [1,2,5] However, congenital urethral strictures are exceedingly rare in infants. [6] The reason for this is that, whatever the etiology of stricture, excessive inflammation in the injury region and increased accumulation of type 3 collagen as a result of this inflammation is considered to cause fibrosis. [7] To date, many agents have been studied to prevent formation of fibrosis, increase success rates of surgical treatments or reduce recurrence; however, none have entered routine use. [8]

Platelet-rich plasma (PRP) is a preparation of autologous plasma enriched with platelet concentrations above that normally found in whole blood. [9] PRP increases the speed of wound healing due to containing many growth factors and it is stated that the synthesis of type 3 collagen causing fibrosis reduces with the effect of these factors. [10-12] Due to this effect of PRP, it is considered that it will have a significant effect on urethral healing. However, there is no information about whether this effect will be enhanced by the easily applied method of instillation, or by submucosal injection of PRP. This study investigated the effects on urethral healing, inflammation and fibrosis of PRP administered as intraurethral instillation and as submucosal injection in an experimentally-induced urethra injury model in rats and compared the administration methods.

The aim of this study is to develop new treatment modalities for urethral stricture that may form after urethral injury by evaluating the effect of PRP and the efficacy of the form of administration of PRP.

Material – Method:

This study was completed in N.E. University Konüdam Experimental Medicine Application and Research Center after receiving permission from the local animal ethics committee.

Rats and Anesthesia

A total of 65 Wistar male albino rats weighing 250-300 g were used in the study. The animals were kept in separate cages at room temperature (22 °C) with 50% moisture during preoperative and postoperative periods. On the day of the experiment, rats were administered anesthesia with ketamine (50 mg/kg) under sterile conditions. Rats other than Wistar Albino species, female rats, those below 250 g and younger than 3 months were not included in the study.

Preparation of platelet-rich plasma (PRP)

Platelet-rich plasma (PRP) was prepared daily from the blood taken from a male (Wistar albino) rat. To take the sample for PRP, blood was taken from the heart under anesthesia and then the animal was euthanized (with the cervical dislocation method). Blood samples were taken in tubes containing sodium citrate (blood/sodium citrate 3.8%=9:1) and gently mixed. Then the first centrifugation procedure (440 xg, 10^{1}, 20 ℃) was completed. The supernatant was obtained, transferred to a new tube and the second centrifugation was completed (800 xg, 12^{1}, 20 ℃). After the second centrifugation, nearly 2 ml of the upper section was removed with a pipette. The remaining PRP was gently mixed and platelet count measurement performed (~$2x10^{6}/mm^{3}$). The measured PRP was used in applications. All procedures were completed under sterile conditions.[13] PRP was prepared fresh before administration and the procedure. The PRP preparation process including taking blood took nearly 60 minutes.

Internal urethrotomy model and PRP application

In the study, a pediatric internal urethrotomy scalpel was used to induce a urethral injury model and a longitudinal 0.5 cm incision was made in 12 o'clock direction from 0.5 cm proximal around the urethra to encompass muscles and corpus spongiosum. Then intraurethral instillation was performed with a 22 Ga catheter sheath in the groups. Submucosal PRP injection was performed with a PPD injector (Fig. 1).

Study Groups

At the beginning of the study, 15 rats were separated for preparation of PRP and PRP was prepared daily. Later, 50 rats were randomized into 5 groups, with 10 rats in each group.

Group 1 was sham group and only underwent daily urethral catheterization,

Group 2 was given instillation of intraurethral 0.9% saline once a day using a 22 Ga catheter sheath (urethral injury + physiologic saline [(UI+PS])

Group 3 was given submucosal injection of PRP after urethral injury (UI+PRPs). Submucosal PRP was injected at 150 microliters (300 million platelets/administration).

Group 4 was given instillation of intraurethral PRP once a day using a 22 Ga catheter sheath after urethral injury (UI+PRPx1). PRP was administered into the urethra of rats as an instillation and one application of 300 million platelets/150 microliters was given for 15-days duration.

Group 5 was given instillation of intraurethral PRP twice a day using a 22 Ga catheter sheath after urethral injury (UI+PRPx2). PRP was administered into the urethra of rats as an instillation and one application of 300 million platelets/150 microliters was given for 15-days duration.

Final Evaluation

On day 15, the penises of the rats were degloved to perform penectomy. Rat penises were placed in 10% formaldehyde and sent to the pathology department for histopathologic analysis. At the end of the study, 1 rat in the sham group (n=9), 3 rats in the UI+PS group (n=7), 3 rats in the UI+PRPs group (n=7), and 1 rat in the UI+PRPx2 group (n=9) died due to anesthesia and environmental factors, so the study was completed with the remaining 42 rats.

Histopathologic Analysis

Histopathologic analysis was performed under light microscope by a single independent pathologist blinded to the study groups. Until the day of macroscopic examination, the urethral tissues were fixed in 10% formalin in a separate dish for each rat. During the macroscopic examination, the tissue samples were cut into squares at 3-mm intervals and embedded in paraffin blocks. Slices of 4-micron thickness were cut from the paraffin blocks and stained with hematoxylin and eosin (HE) and with Masson trichrome for histochemical examination. The preparates were examined under light microscope at x100 and x200 magnification. For the histopathologic examination of the tissues, evaluation was made of spongiofibrosis, inflammation, and congestion in vascular structures. In the histochemical examination, spongiofibrosis was examined with Masson Trichrome staining.

Spongiofibrosis was evaluated as 0 = none; 1 + ≤10% tissues with fibrosis; 2 + = 10%-49% tissues with fibrosis; and 3 + ≥50% tissues with fibrosis.
Inflammation was evaluated as: 0 = none; 1+ = 5-10 lymphocytes/x200 magnification; 2+ = 11-50 lymphocytes/x200 magnification; and 3 + = > 50 lymphocytes/x200 magnification.
Congestion in vascular structures was calculated by counting the number of vessels with congestion in the tissue at each x100 magnification and dividing this by the number of total x100 magnification areas in the tissue: 0: none, 1 + = 1-3, 2 + > 3-6, and 3 + = >6-10. Hyperemia and edema were evaluated according to their presence in biopsy samples (Fig. 2).

While inflammatory cells in subepithelial tissue were assessed for identification of mucosal inflammation, identification of serosal inflammation assessed inflammatory cells found in the tunica adventitia. The results of this assessment identified that rats receiving PRP treatment had fewer inflammatory cells observed in the submucosal area compared to the serosal area.

Statistical Analysis

Statistical analysis was performed with SPSS, v.23.0 statistical software (SPSS, Inc. Chicago, IL, USA). Chi square tests were used to understand if distributions of categoric variables were different across groups. Categoric variables are described by frequencies and percentages. Continuous variables are presented as mean and standard deviations. The Independent samples t-test and Kruskal Wallis test were used for the comparison of continuous variables among groups. A p value <0.05 was considered statistically significant.

Results:

During intraurethral administration, no side effects were observed in rats. During the study 8 rats died due to anesthesia and environmental factors, while 42 rats survived. Penectomies were performed on the 15^{th} day and rats were sacrificed. Forty-two rats were included in the assessment. Rats included in the assessment were evaluated histopathologically for mucosal inflammation, urethral spongiofibrosis, edema, serosal inflammation and congestion. All sections were monitored for mucosal hemorrhage but this was not scored.

Histopathologic assessment was evaluated and compared based on percentages and scoring.

When all groups are compared with each other, the lowest values for mucosal inflammation, spongiofibrosis, edema and serosal inflammation were measured in the sham group, while highest values for mucosal inflammation, spongiofibrosis and edema were measured in the UI+PS (0.9% saline) group. For serosal inflammation, the highest value was measured in the UI+PRPx2 group. When all groups are compared, there were significant differences found for mucosal inflammation, spongiofibrosis and serosal inflammation. No significant difference was identified for edema. The analyses of percentages and scoring for groups are shown in Table 1

The sham group was identified to have lowest score values for all histopathologic parameters. Data for comparisons between the sham group and other groups are shown in Table-2.

The urethral injury+ 0.9% saline (UI+PS) group was observed to have highest values in terms of mucosal inflammation, spongiofibrosis and edema.

When the UI+PS group is compared with the PRP groups, mucosal inflammation and spongiofibrosis were observed to be significantly greater in the UI+PS group. The highest significant difference for spongiofibrosis was identified in the UI+PRPx1 group. There were no significant differences between the UI+PS group and the PRP groups in terms of edema.

When the UI+PS group is compared with the PRP groups in terms of serosal inflammation, serosal inflammation was identified to be significantly less in the UI+PS group compared to all PRP groups.

When the PRP groups (UI+PRPx1, UI+PRPx2, UI+PRPs) are compared with each other, there were no significant differences observed in terms of mucosal inflammation, spongiofibrosis, edema and serosal inflammation. However, the mucosal inflammation and serosal inflammation were observed to be lower in the UI+PRPs group, while spongiofibrosis was less in the UI+PRPx1 group (Table 3).

Urethral congestion was observed to be similar in all groups and no differences were identified between the groups.

Discussion:

Urethra stricture is a disease with high cost disrupting quality of life and with frequent recurrence. There are many factors in the etiology led by trauma, urethra infections, cardiac surgeries, endoscopic interventions or Foley catheter insertion. With the increase in endoscopic treatment especially in recent years, there is an increase in the incidence of iatrogenic urethra stricture. [(14)] After urethra stricture has formed once, it has high recurrence rates and may be treated with repeated minimal invasive procedures, self-dilatation and/or complex surgeries like urethroplasty. [(15-17)] As a result, it is very important to find a medical treatment modality that will prevent formation of urethra stricture and/or lengthen recurrence duration. There is still no medical treatment found in spite of the increase in prevalence of urethra stricture in recent years, the effect on patient quality of life and high cost rates, with experimental applications not going beyond preventive agents. When the literature is examined, many agents have been trialed for prevention of urethra stricture and different administration methods have been used. Administration methods vary according to effect mechanism of the agent; however, to date there is no study found about which administration method and administration frequency is superior for a certain agent. In our study, different to the literature, the efficacy of PRP administration was compared in terms of administration route and dose for the first time. As expected, intraurethral PRP treatment was shown to be protective against urethral stricture, with the pathologic assessment results of the PRP groups determined to be superior to the sham and saline groups.

Though no significant difference was identified for pathologic assessment of the PRP subgroups of UI+PRPx1, UI+PRPx2 and UI+PRPs, interestingly the UI+PRPx1 group had the lowest spongiofibrosis scores. The lowest scores for serosal and mucosal inflammation were identified in the UI+PRPs group. Based on these results, we believe more than one administration of PRP is not superior. The low incidence of spongiofibrosis especially leads to consideration that intraurethral and single-dose administration of PRP may be more effective to prevent urethral stricture.

Urethra stricture was identified to have high recurrence risk due to abnormal fibrosis increase.[7] Studies have observed fibrotic areas causing urethral stricture have 32% increases in type 3 collagen concentrations. [18]

To prevent this abnormal increase in fibrosis, a variety of experimental and clinical studies have been performed about the administration of medications or materials with antifibrotic effect. [19-21] The first studies about prevention of urethra stricture clinically administered steroid treatments like triamcinolone, but were not very successful. [20,22] In rat models, periurethral botox-A injection, mitomycin-c and dexpanthenol instillation were administered and the 3 agents were shown to reduce fibrosis and inflammation. [19,23,24] In fact, mitomycin-c was clinically injected in the submucosa in anterior urethra stenosis and shown to reduce fibrosis. [25] The increasing costs of urethral stricture continue to be a focus of interest currently. In clinical studies, caprotil gel, halofuginone, hyaluronic acid and carboxymethyl cellulose were identified to reduce recurrence of stenosis and postoperative pain when administered as instillations. [21,26,27]

As seen in the literature, clinical and animal studies were performed with many agents and different methods and different administration methods were applied and all were shown to be effective in different studies. However, there is no study showing which administration method is more effective in a single study. Additionally, none of these treatments have entered routine use to prevent urethral stricture.

Platelet-rich plasma (PRP) contains high amounts of growth factor. The most important of these growth factors is platelet-derived growth factor (PDGF), which is a factor repairing connective tissue and initiating wound healing. PDGF is the first factor initiating processes including mitogenesis, angiogenesis and macrophage activation. Many growth factors contained in PRP increase the speed of regenerative processes and reduce inflammatory factors, reducing fibrosis development. Studies with autologous PRP have shown that PRP increases the rate of wound healing, closes wounds more easily in open diabetic wounds and reduces inflammation. Due to these effects, autologous PRP is routinely used in many clinics like plastic surgery and orthopedics. [10-12,17,22-24] Autologous PRP

contains many growth factors like endothelial growth factor, insulin-like growth factor, transforming growth factor and fibroblast growth factor expressed by mesenchymal stem cells. These growth factors prevent excessive accumulation of type 3 collagen in tissues reducing fibrosis development. Linked to the effect of these growth factors, PRP was shown to be effective in prevention of fibrosis following urethral injury. [28,29] Tavukçu et al. administered PRP into the urethras of rats with induced experimental urethra injury and determined that it reduced type 3 collagen synthesis in urethra stenosis and protected against fibrosis. [28] However, there was no study identified performed with PRP or any other agent with different administration methods and numbers of administrations like urethral instillation and submucosal injection to identify the optimal treatment protocol.

This study compared the protective effect of PRP in intraurethral and submucosal administration for urethral stricture developing after iatrogenic induced urethra trauma. In conclusion, PRP was seen to significantly reduce urethral spongiofibrosis independent of form of administration.

In the three PRPs, PRPx1, and PRPx2 groups, spongiofibrosis was identified to be low, with spongiofibrosis score lowest in the PRPx1 group and highest in the PRPs group. Additionally, the PRP group with instillation 1 time per day had lower score values for urethral fibrosis than the PRP group with instillation 2 times per day; however, there was no statistical difference identified. This situation shows that the increase in the number of PRP administrations was not effective for protection from stenosis, and that a single dose was sufficient.

There are a range of studies about wound healing related to PRP. Massara et al. [10] showed PRP has increasing effect on healing in ischemic and diabetic foot wounds. Nikopulos et al. [11] performed a study showing the efficacy of PRP use for pubourethral ligament restoration in stress urinary incontinence. Guinot et al. [12] used platelet-rich fibrin membrane for closure after urethroplasty for distal hypospadias and there are publications about reduced fistula development. Arnalich et al. [30] used solid PRP as OSD in the eye and showed it was an effective and safe preparate for corneal ulcers and perforations in eye surgery. Mohammadi et al. [31] showed effective wound healing in rats.

As seen in these studies, PRP has even been used in the eye and positively affected wound healing and reduced fibrosis due to containing many growth factors without identified side effects. In our study, it was identified to be more effective on fibrosis when administered as urethral instillation.

In this study, the efficacy of PRP administered as intraurethral instillation 1 and 2 times per day and as submucosal injection 1 time per day was compared in rats with urethral injury induced. When compared with intra urethral 0.9% saline instillation after urethra injury, all PRP groups were identified to have significant degrees of reduction in mucosal inflammation and urethral fibrosis.

PRP is a current topic, coming to the fore as a cheap and easily applicable method without autologous side effect profile. In our study, the administration method in rats with experimental urethra injury induced was compared with the UI+PS group. In our study, all administration methods for PRP were identified to be effective, aid in healing urethral tissue and reduce inflammation parameters. When the administration route is compared, administration as urethral instillation 1 time per day was identified to be most effective on spongiofibrosis and reduced it most. As a result, it is considered that this may be chosen for clinical administration due to ease of administration to patients.

In summary, PRP affects synthesis of type 3 collagen especially and increases normal wound healing due to containing many growth factors. It is a simple, cheap and effective preparate with no side effects that can be obtained by autologous means routinely used in other disciplines in medical practice. It may be used as routine instillation to protect against iatrogenic urethra stricture and/or to prevent recurrence after treatment.

PRP administered through intraurethral instillation and submucosal route is a promising preparate that may prevent the development of fibrosis and inflammation as a result of urethral injury and increase the speed of normal urethral healing. However, in our study administration of PRP as instillation 1 time per day was observed to be more effective than submucosal administration and administration as instillation 2 times per day. Administration of PRP as 1 daily intraurethral instillation to protect against urethral stricture may be chosen due to easy application. However, there is a need for clinical and experimental studies about the long-term outcomes to better evaluate the effect of PRP.

Study Limitations:

The most important limitation of the study is the short follow-up period for an animal experiment. This follow-up duration was not sufficiently long to determine fibrosis that may develop in the long term and possible side effects.

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Table-1: Pathologic parameters numbers and ratios between groups. (μ: Chi-Square test)

Pathologic Parameters	**Group-1** Sham n(%)	**Group-2** Urethral injury + Saline Daily (UI+PS) n(%)	**Group-3** Urethral injury + submucozal PRP injection (UI+ PRPs) n(%)	**Group-4** Urethral injury + PRP X1 (UI+PRPx1) n(%)	**Group-5** Urethral Injury + PRP X2 (UI+PRPx2) n(%)	**Total n(%)**	**P Value All Groups$^{\mu}$**
Mucozal Inflamation							**<0,001**
0	2(%22,2)	0	0	0	0	2 (%5)	
1	7(%77,8)	0	7(%100)	6(%60)	6(%66,7)	26(%62)	
2	0	4(%57,1)	0	4(%40)	3(%33,3)	11(%26)	
3	0	3 (%42,9)	0	0	0	3 (%7)	
Spongiofibrozis							**<0,001**
0	3(%33,3)	0	0	0	0	3(%7,1)	
1	3(%33,3)	0	3(%42,9)	8(%80)	4(%44,4)	18(%42,9)	
2	3(%33,3)	4(%57,1)	4(%57,1)	2(%20)	5(%55,6)	18(%42,9)	
3	0	3(%42,9)	0	0	0	3(%7,1)	
Edema							0,627
0	5(%55,6)	3(%42,9)	2(%28,6)	3(%30)	2(%22,2)	15(%35,7)	
1	4(%44,4)	3(%42,9)	5(%71,4)	6(%60)	7(%77,8)	25(%59,5)	
2	0	1(%14,3)	0	1(%10)	0	2(%4,8)	
Serozal Inflamation							**0,003**
0	6(%66,7)	3(%42,9)	0	0	0	9(%21,4)	
1	3(%33,3)	4(%57,1)	4(%57,1)	3(%30)	2(%22,2)	16(%38,1)	
2	0	0	2(%28,6)	4(%40)	4(44,4)	10(%23,8)	
3	0	0	1(%14,3)	3(%30)	3(%33,3)	7(%16,7)	

Table-2: Histopathological scores of groups and total P values (ᵅ Kruskal-Wallis test)

Pathologic Parameters	**Group-1** *Sham (Mean Value ±SD)*	**Group-2** *Urethral injury + SF Daily (UI+SF) (Mean Value ±SD)*	**Group-3** *Urethral injury + submucozal PRP injection (UI+ PRPs) (Mean Value ±SD)*	**Group-4** *Urethral injury + PRP X1 (UI+PRPx1) (Mean Value ±SD)*	**Group-5** *Urethral Injury + PRP X2 (UI+PRPx2) (Mean Value ±SD)*	**P Value** *All Groups*$^{\alpha}$
Mucozal Inflamation	0,77±0,44	2,42 ±0,53	1±0	1,4 ±0,51	1,33±0,50	**<0,001**
Spongiofibrosis	1 ±0,86	2,42 ±0,53	1,57±0,53	1,2 ±0,42	1,55±0,52	**0,004**
Edema	0,44 ±0,52	0,71 ±0,75	0,71±0,48	0,8 ±0,63	0,77±0,44	0,664
Serozal Inflamation	0,33 ±0,50	0,57 ± 0,53	1,57±0,78	2 ± 0,81	2,11±0,78	**<0,001**

Table-3: P values between sham group and experiment groups (*Independent t test)

Pathologic Parameters	Sham Vs UI+PS (p Value)*	Sham Vs UI+PRPx1 (p Value)*	Sham Vs UI+PRPx2 (p Value)*	Sham Vs UI+PRPs (p Value)*
Mucozal Inflamation	**<0,001**	**0,012**	0,024	0,207
Spongiofibrozis	**0,001**	0,524	0,12	0,149
Edema	0,414	0,204	0,165	0,312
Serozal Inflamation	0,375	**<0,001**	**<0,001**	**0,002**

Table-4: P values between experiment groups (*Independent t test)

Pathologic Parameters	UI+PS Vs UI+PRPx1 (p Value)*	UI+PS Vs UI+PRPx2 (p Value)*	UI+PS Vs UI+PRPs (p Value)*	UI+PRPx1 Vs UI+PRPx2 (p Value)*	UI+PRPx1 Vs UI+PRPs (p Value)*	UI+PRPx2 Vs UI+PRPs (p Value)*
Mucozal Inflamation	**0,001**	**0,001**	**<0,001**	0,779	0,061	0,102
Spongiofibrozis	**<0,001**	**0,006**	**0,011**	0,121	0,130	0,953
Edema	0,803	0,836	1,0	0,931	0,768	0,789
Serozal Inflamation	**0,001**	**0,001**	**0,017**	0,766	0,297	0,193