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LARYNGOLOGY

Platelet-rich plasma for laryngotracheal reconstruction: an experimental study

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Abstract

Objectives/hypothesis This study was designed to evaluate the graft healing effect of topical application of platelet-rich plasma (PRP) for laryngotracheal reconstruction (LTR) in a rabbit model.

Study design It is a prospective randomized control animal study.

Materials and methods Sixteen healthy New Zealand White rabbits were assigned to two groups of eight animals each. The control group underwent LTR with anterior auricular cartilage graft. The PRP group underwent the same surgical procedure plus PRP application over the anastomosis and surgical field. Two animals in the PRP group and two animals in control group died due to severe respiratory distress on postoperative days 10, 12, 15, and 18. Six rabbits ($n=3$ for control group and $n=3$ for PRP group) were sacrificed at 4 weeks, and six rabbits ($n=3$ for control group and $n=3$ for PRP group) were sacrificed at 8 weeks. Laryngotracheal regions were evaluated histopathologically.

Results Macroscopically, the average anteroposterior and lateral diameter of the reconstructed region and the degree of lumen patency on postoperative 4th week and 8th week were not statistically different among two groups. There was no significant difference between the groups in terms of any of the microscopic findings when the analysis was made separately. However, analysis of the total number of rabbits has shown that new cartilage formation and angiogenesis were more pronounced in PRP group than control group.

Conclusions Application of PRP contributed to better healing in airway surgery by promoting a release of growth factors that stimulate new cartilage formation and angiogenesis.

Keywords Platelet-rich plasma · Laryngotracheal reconstruction · Wound healing

Introduction

Laryngotracheal stenosis (LTS) is characterized by significant narrowing of airway leading to airway obstruction. Causes of LTS include congenital or acquired factors and autoimmune disorders [1]. Acquired stenosis secondary to prolonged endotracheal intubation is observed in of 0, 24% of the neonatal population. The overall incidence of subglottic stenosis following intubation in the pediatric population is unknown [2]. There is no single method for reconstructing the airway; the management of laryngotracheal stenosis should be patient-specific and each patient generally requires a combination of different techniques. The preferred reconstructive technique depends on the level of obstruction and the etiology of the stenosis. Laryngotracheal reconstruction (LTR) with anterior or posterior costal cartilage grafting is the mainstay open procedure for the repair of moderate to

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severe LTS. The aims of LTR are to expand the subglottic framework with elliptically shaped free cartilage grafts placed into the anterior or posterior part of the cricoid cartilage or both.

Many different materials have been used as grafts for laryngotracheoplasty. The ideal laryngotracheal graft needs to be responsive, easy to carve and shape. It should contour the airway lumen and retain architectural integrity to hinder prolapsing. The main source for cartilage is the rib for pediatric laryngotracheoplasty [3]. Even though laryngotracheal surgery success rates can be as high as 90%, recurrent airway narrowing resulting from an unsuccessful surgery can be devastating for patients [4].

Proper wound healing is believed to be an important factor in preventing restenosis [5, 6]. An obvious inflammatory reaction that causes granulation tissue and a collagen deposition leads to scar tissue causing restenosis. The direct application of growth factors to the reconstructed region can contribute to the controlling of the inflammatory response and promote wound healing within the airway [6]. A surgical method that could use a growth factor's ability to accelerate healing process after airway reconstruction may be used for patients with laryngotracheal stenosis.

Marx was the first to report on platelet-rich plasma (PRP), a first-generation platelet concentrate [7]. PRP is readied by centrifuging blood with an anticoagulant, intensifying the platelet fraction. PRP has been shown to have clinical application in vocal-fold scar treatment, tympanoplasty and nasal mucosal healing [8].

The therapeutic effects of PRP are supposed to result from high levels of growth factors such as transforming growth factor b1 (TGF-b1), platelet-derived growth factor (PDGF), vascular endothelial growth factor, fibroblast growth factor 2 (FGF-2), and insulin-like growth factor 1 (IGF-1) [9]. From the literature, it is obvious that PRP has a positive effect on cartilage repair [10, 11].

On the basis of this regenerative characteristic of PRP, we investigated the graft healing effect of PRP on the study group and compared it to control group in a rabbit model of LTR. We hypothesized that topical application of autologous PRP can be an alternative treatment that enhances normal wound healing within the airway.

Materials and methods

Animals and study design

Sixteen white New Zealand adult male rabbits weighting between 2500 and 3500 g were used with approval of the Laboratory Animals Local Ethics Committee. Surgical procedures, as well as preoperative and postoperative care were performed in accordance with the ethical standards. The rabbits were randomly assigned into two groups of eight animals each: a PRP group and a control group. The control group underwent LTR only and PRP group underwent LTR with PRP application to the grafting field.

Surgical procedure

The rabbits received intramuscular anesthesia with ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (7 mg/kg) without intubation. The rabbit was placed in a supine, hyperextended position on a shoulder roll. The left ear and neck were shaved and sterilized with a mixture of iodine and 70% ethanol solution. 2 mL of 1% lidocaine hydrochloride was injected subcutaneously into the surgical area to provide analgesia.

A transverse incision was made over the dorsal surface of the left ear. A 15×10 mm section of cartilage was harvested with the perichondrium intact on the dorsal surface. The cartilage grafts were soaked in 1 ml PRP solution. A 4-0 Vicryl suture was used to close the ear skin incision. Then, a 3-cm vertical midline incision was made in the anterior neck. The anterior cervical strap muscles were retracted laterally to expose the laryngotracheal segment. An anterior, midline laryngofissure was performed through the cricoid cartilage and the first two tracheal rings. 15×5 mm full-thickness surgical defect was created (through cartilage and mucosa) in the anterior cricoid cartilage and the first two tracheal rings. Reshaped auricular cartilage graft was positioned with the perichondrium facing the airway lumen between the cut edges of the divided cricoid and tracheal cartilage and secured with 5-0 Vicryl sutures (Fig. 1). In the PRP group ($n = 8$), after suturing the cartilage graft onto the defect, 1 ml PRP was injected along the resected margins of the anastomosis. An air-leak test was carried out with saline in an effort to confirm order graft security. The strap muscles and skin were closed in layers with 4-0 Vicryl sutures, and the rabbits were carefully observed daily for evaluation of infection, local reactivity and signs of respiratory distress.

Postoperatively, the rabbits were administered 2 mg dexamethasone to treat airway edema, 20 mg/kg amoxicillin for antimicrobial prophylaxis and 3 mg/kg tramadol for analgesia for 3 days.

Two animals in the PRP group and two animals in control group died due to severe respiratory distress on postoperative days 10, 12, 15 and 18. Six rabbits ($n = 3$ for control group and $n = 3$ for PRP group) were sacrificed at 4 weeks, and six rabbits ($n = 3$ for control group and $n = 3$ for PRP group) were sacrificed at 8 weeks. The rabbits were sedated and humanely euthanized by an intracardiac injection of 150 mg/kg sodium phenytoin. All larynges were harvested and placed in formaldehyde fixative. The harvested specimen was photographed and then embedded in plastic for histological analysis.

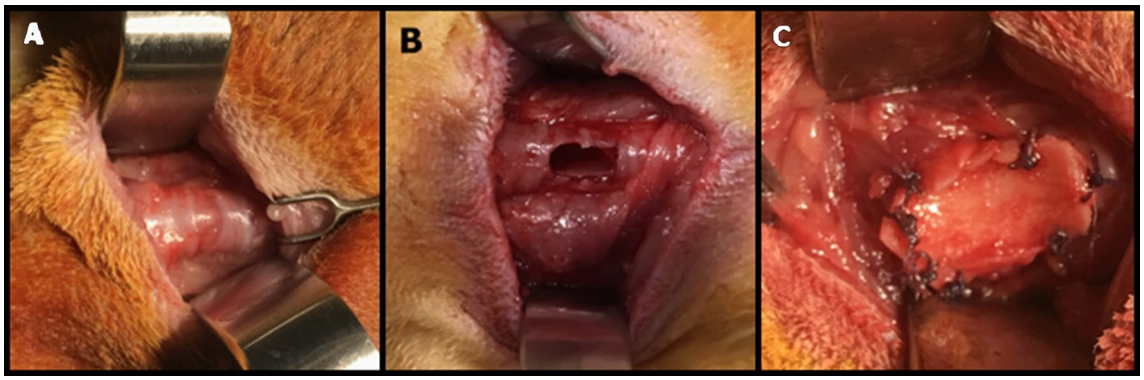

Fig. 1 Laryngotracheal complex of the rabbit exposed **a** before and **b** after creation of the defect. An incision is made through the cricoid cartilage and first and second tracheal rings. **c** After reconstruction with anterior auricular cartilage graft. Graft is well seated on to the surgical defect

Preparation of platelet-rich plasma

A 10 ml syringe containing 1,5 ml sodium citrate was used to collect 8 ml whole blood from the marginal auricular vein of each rabbit. The mixed solution was centrifuged at a speed of 300×*g* for 10 min at 4 °C in order to separate the plasma from the red blood cells. Then, the plasma was transferred to a new sterile tube and centrifuged at a speed of 400×*g* for 20 min at 4 °C. Following centrifugation, the resulting 2 ml PRP was obtained from each rabbit. Then, rabbits were treated with their own PRP [12].

Macroscopic and histological assessments

The harvested larynges were examined macroscopically to note the anteroposterior and lateral diameters. The luminal obstruction was graded as follows; three for patent airway (0–50%), two for moderately obstructed airway (51–75%), and one for obstructed airway (76–100%). Then, sections were taken from the level of the graft, embedded in paraffin and cut to a thickness of 4 µm. Slides were stained with hematoxylin and eosin (H&E). The specimens were examined on a light microscope (Leika DM6000B; Hamburg, Germany) to evaluate integration of the graft and the surrounding tissues as well as vascularization and viability of the mucosa. The same blinded pathologist examined all the sections. Presence of inflammation (inflammatory cellular invasion and number) (0 none, 1 mild, 2 moderate, 3 severe), angiogenesis (0 none, 1 mild, 2 moderate, 3 severe), foreign body reaction (0 none, 1 mild, 2 moderate, 3 severe), necrosis (0 none, 1 present), new cartilage formation (0 none, 1 present), fibrosis (0 none, 1 present) and granulation (0 none, 1 mild, 2 moderate, 3 severe) were noted by using a grade system under light microscope as described by Yener et al. [13].

Statistical analysis

The data were statistically analyzed using Mann–Whitney *U* and Chi square test. *p* values of ≤ 0.05 were regarded as statistically significant.

Results

In total, sixteen rabbits underwent anterior graft LTR using autografted auricular cartilage. Twelve animals survived during the study period. Complications such as bleeding, infection, subcutaneous emphysema and immediate dyspnea were not observed after the surgical procedures. Two animals in PRP group and two animals in control group died due to a large inflammatory granulation reaction on postoperative days 10, 12, 15 and 18. Histological analysis of dying rabbits confirmed that slippage of the implanted grafts from within the surgical defect caused the inflammatory granulation reaction. These rabbits were not included in the final analysis.

Macroscopic findings

The macroscopic findings are summarized in Table 1. Macroscopically, the grafted region healed well in all 12 rabbits included in the final analysis. The average anteroposterior and lateral diameter of the reconstructed region and the degree of lumen patency on postoperative 4th week was not statistically significantly different in two groups (*p*: 0.376; 0.87; 1; respectively). Similarly, there was no significant difference between the average anteroposterior and lateral diameter of the reconstructed region and the degree of lumen patency on postoperative 8th week (*p*: 0.456; 0.456; 0.817; respectively).

Microscopic findings

The microscopic findings are summarized in Table 2. There was no significant difference between the groups in terms of any of the microscopic findings when the analysis was made separately. However, analysis of the total number of rabbits has shown that angiogenesis was statistically significantly different between the two groups ($p = 0.014$). New cartilage formation was also more pronounced in PRP group (0.83 ± 0.4) than control group (0.1 ± 0.4) ($p = 0.021$). Inflammation and granulation was the same in the groups. Foreign body reaction was 1.16 ± 0.75 in the PRP group and 1.83 ± 0.98 in the control group ($p = 0.343$). In the evaluation of necrosis, fibrosis and granulation, there was no statistically significant difference between the two groups (Figs. 2, 3).

Table 1 Comparison of the macroscopic findings of the platelet-rich plasma (PRP) and control groups

Groups	Ant-post diameter	Lateral diameter	Lumen obstruction
PRP (4 weeks)	7 ± 1	6.3 ± 1.5	0.6 ± 0.5
Control (4 weeks)	5 ± 2.6	7 ± 1	0.6 ± 0.5
p value	0.376	0.87	1
PRP (8 weeks)	7.3 ± 1.5	5.6 ± 0.57	0
Control (8 weeks)	7.3 ± 0.5	5.3 ± 0.57	0.3 ± 0.5
p value	0.456	0.456	0.817
PRP (total)	0.65 ± 1.6	6 ± 1.09	0.33 ± 0.51
Control (total)	0.61 ± 2.1	5.8 ± 0.75	0.5 ± 0.54
p value	0.62	0.90	0.575

Table 2 Comparison of the microscopic findings of the platelet-rich plasma (PRP) and control groups

Groups	Inflammation	Angiogenesis	FBR	Necrosis	NCF	Fibrosis	Granulation tissue
PRP (4 weeks)	2 ± 1.7	1.3 ± 0.5	1.3 ± 1.1	0.33 ± 0.57	0.66 ± 0.5	0.66 ± 0.5	1.66 ± 2.08
Control (4 weeks)	2 ± 1.6	0.33 ± 0.5	2.3 ± 1.1	0.66 ± 0.57	0	1 ± 1	1.66 ± 1.5
p value	0.8	0.5	0.11	0.41	0.83	0.513	1
PRP (8 weeks)	0.6 ± 1.1	1	1	0	1	0	0.33 ± 0.57
Control (8 weeks)	0.6 ± 0.5	0.33 ± 0.5	1.3 ± 0.5	0	0.33 ± 0.57	0.33 ± 0.57	0.33 ± 0.57
p value	0.18	0.22	0.27	1	0.83	0.27	1
PRP (total)	1.33 ± 1.5	1.16 ± 0.75	1.16 ± 0.75	0.16 ± 0.4	0.83 ± 0.4	0.33 ± 0.5	1 ± 1.54
Control (total)	1.33 ± 1.3	0.33 ± 0.5	1.83 ± 0.98	0.33 ± 0.51	0.1 ± 0.4	0.66 ± 0.81	1 ± 1.26
p value	0.362	**0.014**	0.343	0.505	**0.021**	0.565	0.504

Bold value represents $p < 0.05$

FBR foreign body reaction, *NCF* new cartilage formation

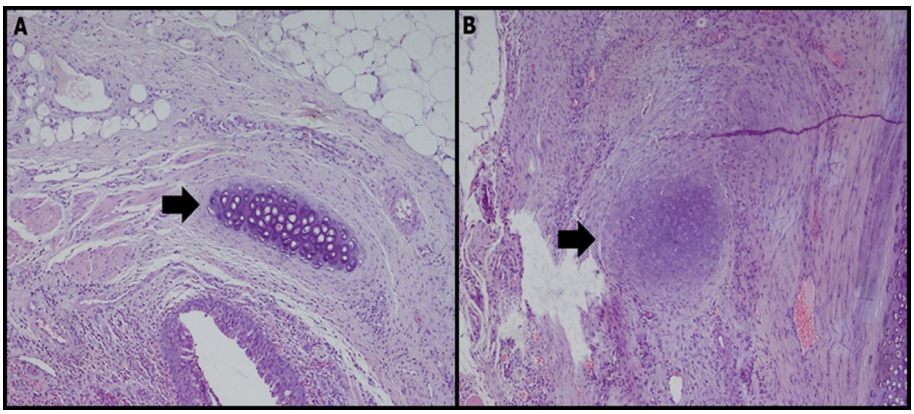

Fig. 2 Laryngotracheal sections at the reconstruction field on the 8th postoperative week: histological specimens were stained with hematoxylin and eosin, ×400 magnification. **a** New cartilage formation in the control group (black arrow) is significantly less as compared to the PRP group and **b** new cartilage formation (black arrow) in the platelet-rich plasma (PRP) group

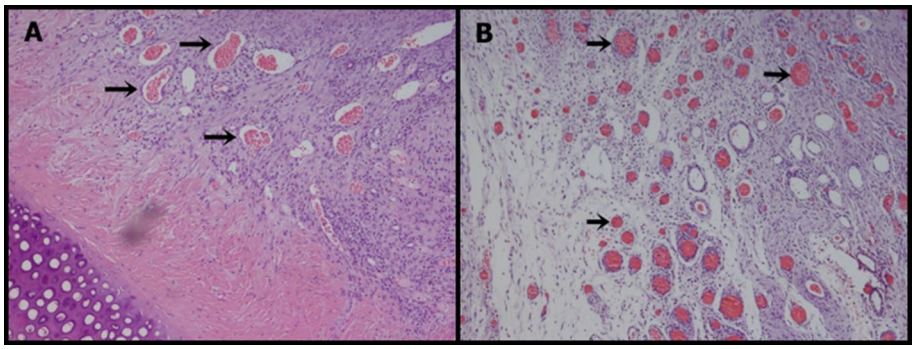

Fig. 3 Laryngotracheal sections in the surgical field on the 8th postoperative week: histological specimens stained with hematoxylin and eosin, ×100 magnification. **a** Section from an animal in the control group (black arrow). Vascularization is significantly less in the basal layer in the control group as compared to the platelet-rich plasma (PRP) group. **b** PRP group exhibits more significant proliferation of vessels in the basal layer (black arrow)

Discussion

Laryngotracheal reconstruction (LTR) with costal cartilage graft is a common surgical option for severe subglottic stenosis. Although the results differ due to the grade of stenosis, a recent meta-analysis has shown an overall decannulation rate of 93.2% for single stage LTR vs 83.7% for double stage LTR [14]. Critical to the success of both of these techniques is the maintenance of low inflammatory response and necrosis of costal cartilage graft [5]. One hypothesis is that growth factors and extracellular matrix play significant roles in determining the outcome of the wound healing process and cartilage graft necrosis due to poor revascularization, leading to scar formation, and restenosis [6]. A surgical method that would enable enhanced wound healing could lead to more favorable surgical results. The present study investigated the effectiveness of platelet-rich plasma (PRP) in wound healing following laryngotracheal reconstruction with anterior cartilage graft through histomorphometric evaluations. The most promising results from this study were that angiogenesis and new cartilage formation was more pronounced in PRP group than control group. It was also noted that inflammation and granulation was the same in PRP and control group.

Although autologous cartilage grafts from the ribs is the most employed, several investigators have looked at the use of homologous cartilage, alloplastic materials, or autologous tissue-engineered cartilage. Gilpin et al. used autologous chondrocytes to fabricate an implantable sheet of cartilage noted and that autologous tissue-engineered cartilage can be used as a graft for LTR [15]. Goldstein et al. [16] produced a custom-designed, 3D-printed, tissue-engineered graft for airway reconstruction and tested it in a rabbit model of LTR. Their findings confirmed that the graft maintains its cartilaginous properties in vitro and in vivo and may favorable in airway reconstruction. Yener et al. used titanium mesh used in laryngotracheal reconstruction of rabbits. They concluded that titanium mesh can be reliably used in cases where more rigid stability is necessary like tracheomalacia [13]. However, it is too early to suggest that this type of composite graft is ready for clinical practice; especially, in cases where large and circular defects need reconstructing.

Platelet-rich plasma is the prepared by enriching autologous plasma with a platelet concentration above that which is found in whole blood. The effectiveness of PRP in promoting healing is significant for tendons, ligaments, and cartilage. Here the repair process tends to be slow due to slow turn over and limited blood supply [17, 18]. Wound healing occurs in three phases: inflammation, proliferation, and remodeling. The inflammation phase is signified by the occurrence of hemostasis, where platelets establish clot formation, and growth factors are released to assist in the activation and attraction of inflammatory cells like neutrophils and macrophages to the injury's location. The proliferation phase is signposted by the construction of an extracellular matrix associated with granulation, contraction and epithelialization [19, 20]. Finally, the production of collagen and scar tissue occurs in the remodeling phase. The progression through the different stages of wound healing is conducted by growth factors and cytokines, many of which are released and regulated by blood components of PRP [20].

PRP has been shown to result in acceptable positive outcomes for various clinical conditions [8, 21]. Dual-speed centrifugation can be utilized to rapidly prepare autologous PRP (in approximately 12 min) during induction of anesthesia and applied without the use of additives as suggested by El-Anwar et al. [22] for myringoplasty surgery. Cobden et al. investigated the effect of platelet-rich plasma on vocal fold scar healing. They concluded that platelet-rich plasma elevated EGF receptor, FGF, and fibroblast growth factor receptor 1 (FGFR-1) levels in the healing vocal fold and had a beneficial effect on the vocal

fold [23]. Similar to our study, Lee et al. [24] recently evaluated the effects of applying platelet-rich plasma jelly over the tracheal anastomosis and surgical field and they found that autologous PRP jelly contributed to better healing of tracheal reconstruction, however, the investigators did not evaluate the use PRP jelly for grafts. Gomes-Caro et al. investigated whether PRP promotes healing and reduces anastomotic complications following airway surgery in a pig model. They found that platelet concentration generates growth factor release within 6 h, triggering the healing process, and promoting angiogenesis in high-risk long tracheal resections, and concluded that autologous PRP may accelerate wound healing of tracheal anastomosis [25]. These findings support our study. In the present study, we observed that angiogenesis and new cartilage formation in laryngotracheal reconstruction was aided by PRP treatment in a rabbit model. On the basis of the criteria used in our study autologous PRP contributed to better healing of airway surgery.

In our study, auricular cartilage was used as a graft material in the rabbit model. A recent systematic review of simulated laryngotracheal reconstruction animal models determined that the rabbit, sheep, and pig models seemed to demonstrate the greatest potential for use as advanced pediatric airway surgery simulation models [26]. Heatley et al. investigated the rate of epithelialization and the survival of cartilage in LTR and directly compared auricular and costal cartilage grafts in a rabbit model. They found no difference in the survival of the two types of cartilage grafts [27]. Using an auricular cartilage graft has many theoretical advantages compared with rib cartilage graft, such as more easier surgery technique and less complication rate.

In the present study, four animals died due to a large inflammatory granulation reaction. Histological analysis of dying rabbits confirmed that slippage of the implanted grafts from within the surgical defect caused the inflammatory granulation reaction. There are a number of options that may assist in preventing the slippage of implanted grafts. This study did not feature the intubation of rabbit nor were the grafts sutured over a rigid framework, such as an endotracheal tube (ET). The use of stents, including an ET, tracheostomy tube, or even a bioresorbable plate can assist in reducing the initial load placed on grafts as the wound heals.

Among the limitations of our study, we include the close period of 4 weeks for animal sacrifice, followed by 8 weeks for the rest of the group. This separation resulted in fewer cases for analysis within the parameters of the mentioned time periods. However, the clinically accepted healing time for airway surgery is 3–4 weeks, and this separation would allow us to analyze healing and neoangiogenesis in different time periods [25].

Conclusion

Autologous PRP application is a feasible and reliable treatment that is not aligned with any negative or systemic effects. Our findings suggested that autologous PRP contributed to better healing in airway surgery. Based on our study, adequate wound healers are expected to sustain positive improvements in airway grade to achieve decannulation, requiring the need for fewer reconstructive surgical procedures.

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Compliance with ethical standards

Conflict of interest We have no potential or competing interests with the content of our paper.

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