

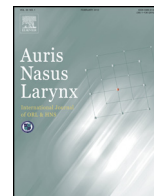


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Can tissue adhesives and platelet-rich plasma prevent pharyngocutaneous fistula formation?

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ABSTRACT

Objective: One of the frequently encountered disorders of wound healing following laryngectomy is pharyngocutaneous fistula. However, although studies have been performed with the aim of prevention of pharyngocutaneous fistulae, there are very few studies with tissue adhesives and platelet-rich plasma. In this study, our aim was to investigate the histopathologic changes in wound healing caused by various tissue adhesives and platelet-rich plasma, together with their effects on prevention of pharyngocutaneous fistula.

Methods: 40 male rats were randomly divided into five groups: control, platelet-rich plasma, fibrin tissue adhesive, protein-based albumin glutaraldehyde and synthetic tissue adhesive groups. The pharyngotomy procedure was performed and was sutured. Except the control group, tissue adhesives and platelet-rich plasma were applied. Then, the skin was sutured. On the seventh day, the rats were sacrificed. The skin was opened and pharyngotomy site was assessed in terms of fistulae. The pharyngeal suture line was evaluated histopathologically by using Ehrlich Hunt scale.

Results: Inflammatory infiltration was found to be higher in “platelet-rich plasma” group than “fibrin tissue adhesive” and “synthetic tissue adhesive” groups. The fibroblastic activity of “platelet-rich plasma”, “fibrin tissue adhesive” and “protein-based albumin glutaraldehyde” groups was higher than the control group. The positive changes created by platelet-rich plasma and fibrin tissue adhesive at the histopathologic level were found together with no detected fistula. Among the study groups, there was no statistical difference for pharyngeal fistula development. This result may be obtained by the small number of animal experiments.

Conclusion: These results shed light on the suggestion that platelet-rich plasma and fibrin tissue adhesive can be used in clinical studies to prevent pharyngocutaneous fistula.

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1. Introduction

One of the frequently encountered disorders of wound healing following laryngectomy is pharyngocutaneous fistula formation. Conditions such as preoperative radiotherapy, history of previous tracheostomy, low blood concentrations of hemoglobin and albumin, positive surgical margins, type of the utilized surgical suture material, and presence of gastroesophageal reflux are

considered as risk factors for pharyngocutaneous fistula [1,2]. Following laryngectomy, risk of pharyngocutaneous fistula ranges between 5 and 65% in primary surgery and 14 and 61% in salvage surgery [2–4].

During total laryngectomy procedure, when there is sufficient mucosa, pharyngeal closure is performed by direct repair, creating minimum tension. Pharynx is closed with three layers of sutures, consisting of mucosa, submucosa and muscle. However, despite existence of sufficient mucosal tissue and satisfactory surgical technique, pharyngocutaneous fistula may still develop [1]. Even with the development of new suture techniques, risk of pharyngocutaneous fistula is still a significant problem. Pharyngocutaneous fistula is initiated by salivary leakage from the

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pharyngeal closure site, followed by the accumulation of this leakage material between the pharynx and skin. This complication increases morbidity, prolongs the length of hospital stay, can create additional requirements for surgery and increases costs [5,6]. Furthermore, it may even cause mortality by leading to carotid rupture and aspiration pneumonia [5,6]. For this reason, research is ongoing to prevent the formation of pharyngocutaneous fistula [7–9]. In the last 15 years, many studies have been performed on the use of various tissue adhesives in esophageal, gastric, colonic, intestinal and pancreatic anastomoses [10–13]. However, although studies have been performed with the aim of prevention of pharyngocutaneous fistulae, there are very few studies with the newly developed tissue adhesives [14,15]. The pharyngeal tract is stratified squamous non-keratinizing type epithelium and has different histological character compared to the gastrointestinal tract. The pharynx lacks muscularis mucosae and submucosa layers which is different from the other gastrointestinal parts. The efficacy of different tissue adhesives and platelet-rich plasma is compared in pharyngeal tract as new data. A point to be especially mentioned is the lack of studies on prevention of development of pharyngocutaneous fistula with platelet-rich plasma in the literature.

In this study, our aim was to investigate the histopathologic changes in wound healing caused by various tissue adhesives and platelet-rich plasma, together with their effects on prevention of pharyngocutaneous fistula.

2. Material and method

Following approval by the Animal Ethics Committee, an experimental study was conducted prospectively. Administration of the pharmaceuticals and obtaining related tissue samples were carried out at the Experimental Animal Research Laboratory.

2.1. Animals

In the study, 40 female rats, 4–6 months of age, with weight of 350–400 g, were used. The rats were randomly divided into five groups of eight. During the study period, rats were kept at room temperature (20 ± 2 °C) in 12 h cycles of daylight/dark environments, fed with standard food pellets and allowed free access to water.

2.2. Anesthesia

The applications were performed under intraperitoneal ketamine/xylazine (50 mg/kg + 5 mg/kg) anesthesia.

2.3. Surgical procedure

The pharyngotomy procedure was performed by the method of Liu et al. [14,15]. After shaving the cervical midline site, the rat was placed in supine position on the operating table and following skin disinfection, a 2-cm vertical incision was made at the cervical skin by a sterile 10# scalpel blade. After lateral retraction of sternocleidomastoid muscle, exposure of trachea and esophagus was obtained. Simultaneously, a metal oral gavage-feeding needle was introduced into the pharynx to be used as a guide. Pharyngeal mucosa was identified and after making a 1-cm pharyngeal incision, from hyoid bone to the cricoid cartilage level, it was sutured by using 3/0 polyglactin 910 (Vicryl, Ethicon Inc., Somerville, NJ, USA). In all groups except the control group, tissue adhesives and platelet-rich plasma were applied, widely covering the entire suture line. Then, the skin was sutured.

2.4. Used materials

2.4.1. Fibrin tissue adhesive (Tisseel®)

It is a steam-sterilized, 2-component fibrin tissue adhesive. It consists of four vials, two of which are lyophilized (adhesive protein concentrate and thrombin) and the other two are diluents (fibrinolysis inhibitor solution and calcium chloride solution). Vials 1, 3 and 4 are obtained from pooled human plasma and vial 2 is of bovine-origin [16].

2.4.2. Protein-based albumin glutaraldehyde (BioGlue®)

This biological protein matrix consists of 10% glutaraldehyde and 45% bovine serum albumin solution [17].

2.4.3. Synthetic tissue adhesive (CoSeal®)

It is fully synthetic and independent of the coagulation cascade. It consists of two different tetra-functional polyethylene glycol macromeres which cross-link with tissue proteins just in seconds [18].

2.4.4. Platelet-rich plasma

In order to prepare platelet-rich plasma, blood was obtained by intracardiac intervention from two rats outside the study group. The ideal approach would be preparing the plasma by using its own blood. However, it was not preferred to do so, since 1.5 ml blood sample, which would be necessary for preparation, would have increased the mortality during the following surgical intervention. The blood samples were withdrawn into anticoagulant tubes including acid citrate dextrose (ACD), and were shaken for 10 s to provide mixing. When the tube was centrifuged at low rpm (200/min, 20 °C, 10 min), three parts were identified. The lowest part belonged to erythrocytes, the middle part was a platelet-leukocyte mixture named *buffy-coat* and the highest part belonged to plasma. When *buffy-coat* and platelet-poor plasma, taking place at the top, were re-centrifuged together (300/min, 20 °C, 15 min), platelet-rich plasma was obtained. It was activated by addition of thrombin and calcium chloride [19].

2.5. Study groups

Group 1 (platelet-rich plasma): Following the surgical procedure, pharynx was repaired primarily with absorbable sutures and “platelet-rich plasma” was applied upon the suture line.

Group 2 (fibrin tissue adhesive): Following the surgical procedure, pharynx was repaired primarily with absorbable sutures and “fibrin tissue adhesive” (Tisseel®, VH, Baxter AG, Vienna, Austria) was applied upon the suture line.

Group 3 (protein-based albumin glutaraldehyde): Following the surgical procedure, pharynx was repaired primarily with absorbable sutures and “protein-based albumin glutaraldehyde” (BioGlue®, CryoLife International®, Inc.; Kennesaw, Ga) tissue adhesive was applied upon the suture line.

Group 4 (synthetic tissue adhesive): Following the surgical procedure, pharynx was repaired primarily with absorbable sutures and “synthetic” (CoSeal®, VH, Baxter AG, Vienna, Austria) tissue adhesive was applied upon the suture line.

Group 5 (control): Following the surgical procedure, pharynx was repaired primarily with absorbable sutures and no material was applied upon the suture line.

2.6. Assessment

2.6.1. Pharyngeal fistula

After surgery, all groups were followed up in terms of development of a pharyngeal fistula for 7 days. On the seventh day of wound healing, the rats were sacrificed by cervical dislocation

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