Comparative aspects of blood vessel and duct branching in rabbit and human submandibular glands

Akimichi Takemura¹, Mamoru Uemura¹, Fumihiko Suwa¹, Lei Zhang², Guang-Yan Yu² and Yi-Ru Fang³

¹Department of Anatomy and ²Department of Oriental Dental Medicine, Osaka Dental University, 8-1 Kuzuhahanazono-cho, Hirakata, Osaka 573-1121, Japan and ³Department of Oral and Maxillofacial Surgery, Peking University School of Stomatology, 22 South Avenue Zhongguancun Haidian District, Beijing 100081, China

We investigated the divergence point, path, number, and thickness of arterial, venous and ductal branches in rabbit and human submandibular glands using the acrylic resin injection method. The results for the two species were then examined and compared. Ten submandibular glands were obtained from five rabbits. Eight submandibular glands were removed from eight Chinese patients who underwent functional neck dissection for primary oral squamous cell carcinoma. The rabbit and human submandibular gland corrosion casts were examined with a stereoscopic microscope. Digital images were captured and analyzed with imaging analysis/measurements software.

The main arterial, venous and ductal stems reached almost to the center of the gland while decreasing in diameter. The first branches diverged from each main stem at the same points like a tree, and ran parallel to each other in both the rabbits and humans. The second branches diverged from each first branch in the same region like a tree, and ran parallel to each other in both the rabbits and humans. The third branches diverged from the each second branch in a similar pattern for both species. The first, second and third branches of the arteries, veins and ducts all diverged at the same points and ran parallel to each other in both the rabbit and human submandibular glands. The locations of divergence and the course of the arterial, venous and ductal branches in the rabbit submandibular gland were thus extremely similar to those in humans. (J Osaka Dent Univ 2012; 46: 221–227)

Key words: Comparative anatomy; Submandibular gland; Rabbit; Human; Plastic injection method

INTRODUCTION

Although the blood supply to rabbit submandibular glands has been studied, branching of the arteries, veins, and ducts has not been reported yet. We investigated the divergence point, path, number, and thickness of the branches of the arteries, veins and ducts in rabbit and human submandibular glands using the acrylic resin injection method. The results were then examined and compared for both species. Partial autologous microvascular submandibular gland transfer is a new treatment method for severe cases of keratoconjunctivitis sicca; its effectiveness, however, has not been confirmed. Therefore, we also considered whether a rabbit would be an appropriate model to study this transplant based on our direct comparisons of branching in rabbit and human submandibular glands.

MATERIALS AND METHODS

Materials

Rabbit

Ten submandibular glands were obtained from five Japanese white rabbits with an average weight of 3.2 kg (Hamaguchi Laboratory Animals, Hyogo, Japan). All procedures were approved by the Osaka Dental University Animal Research Committee (08-02001, 09-02001) and performed in accordance with guide-
lines for animal experiments.

**Human**

Eight submandibular glands were removed from eight Chinese patients (five males and three females with a mean age of 53 years) who underwent functional neck dissection for primary oral squamous cell carcinoma. Human tissue was used with informed consent from the patients and approval by the Peking University School of Stomatology Local Ethics Committee (IRB 00001050–08048).

**Methods**

**Preparation of the rabbit specimens**

Under inhalation anesthesia with isoflurane (Forane®; Abbott Japan Co., Ltd., Tokyo, Japan), the rabbits were intravenously injected with 5 mL heparin sodium (Novo Heparin Injection 5000®; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). After 30 minutes, they were euthanized with an intravenous injection of excess sodium pentobarbital (Nembutal®; Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). Physiological saline was infused into the right and left common carotid arteries to remove the blood. The plastic injection method was used to observe the stereoscopic construction of blood vessels and ducts in the glands.²³ Red acrylic resin colored with red pigments (Cromophtal Red; Ciba Japan K.K., Tokyo, Japan) was injected into the common carotid arteries, blue acrylic resin colored with blue pigments (Cromophtal Blue; Ciba Japan K.K.) into the internal jugular veins, and yellow acrylic resin colored with yellow plastic paints (Oil Base Silicon for Metal Courting; Asahipen Corp., Osaka, Japan) into the submandibular ducts. After hardening the resin under warm water, specimens were soaked in 10% sodium hydroxide and washed under running water to remove the soft tissue. Ten corrosion casts were prepared.

**Preparation of the human specimens**

Blood was removed from the obtained submandibular glands with physiological saline infusions. To observe the stereoscopic arrangement of the arteries, veins and ducts, red acrylic resin (Red Methacrylate Solution®; Beijing Latex Factory, Beijing, China) was injected into the arteries, blue acrylic resin (Blue Methacrylate Solution®; Beijing Latex Factory) into the veins, and white acrylic resin (White Methacrylate Solution®; Beijing Latex Factory) into the ducts. Specimens were soaked in 10% sodium hydroxide and washed under running water to remove the soft tissue. Eight corrosion casts were prepared.

**Measurements of the size of the submandibular gland**

The length and width of rabbit submandibular glands were measured using calipers (Pocket Caliper; Shinwa Rules Co., Ltd, Niigata, Japan). The size of human submandibular glands were based on measurements by Xu et al.²⁵

**Branching of the submandibular gland arteries, veins and ducts**

The rabbit and human submandibular gland corrosion casts were examined with a stereoscopic microscope (Stemi 2000–C; Carl Zeiss Japan, Tokyo, Japan) to investigate the arterial branches, venous branches and submandibular ducts. Digital images were captured and analyzed with imaging analysis/measurement software (Image Pro Plus 5.0 J®; Nippon Roper, Tokyo, Japan). The arterial and venous submandibular gland branches and submandibular gland duct that passed through the hilum of the submandibular gland were designated as main stems, down to the point where their thickness decreased by half. Thinner branches that diverged from each main stem were designated as first branches, down to the point where their thickness decreased by half. Branches that extended from the main stem with a thickness half the size of the main stem were also designated as first branches. Thinner branches that diverged from each first branch were designated as second branches, and thinner branches that diverged from each second branch were designated as third branches. The number of blood vessels and ducts were counted for each branch type using the image analysis software, and their diameters were measured. Average values are represented as mean and standard deviation.
RESULTS

Rabbit submandibular gland (Figure 1)
The mean length of the rabbit submandibular gland was 15.80 ± 1.55 mm, and the mean width was 9.15 ± 0.75 mm. The main stem of the arterial branch had a mean diameter of 522.60 ± 88.36 μm. The main stem of the venous branch had a mean diameter of 871.71 ± 184.60 μm, and the submandibular duct a mean diameter of 434.08 ± 60.52 μm. All three stems entered at the hilum of the submandibular gland and ran parallel to each other. The divergence point, path, number, and thickness of the blood vessel and duct branches were as follows:

Arterial branches
The main stem reached almost to the center of the gland while decreasing in diameter. Five to seven first branches with an average diameter of 257.72 ± 32.83 μm diverged from the main stem like a tree staggered along its length. Each first branch separated into 4 to 7 second branches with an average diameter of 174.99 ± 22.58 μm, also in a tree-like pattern staggered along its length, and each second branch separated into 4 to 6 third branches with an average diameter of 89.35 ± 17.6 μm in a similar pattern. The number and dimensions of the branches are shown in Table 1.

Venous branches
The main stem reached almost to the center of the gland while decreasing in diameter. An average of 4 to 6 first branches with an average diameter of 430.49 ± 114.84 μm diverged from the main stem like a tree staggered along its length. These first branches diverged at the same point on the main stem as the arterial first branches, and ran parallel to them. Each first branch separated into 4 to 6 second branches with an average diameter of 189.42 ± 35.22 μm in a tree-like pattern staggered along its length. These second branches diverged from the same location on the first branches as the arterial second branches and ran parallel to them. Each second branch separated into 3 to 5 third branches of average diameter 98.65 ± 22.39 μm in a similar pattern. These third branches diverged from the same location on their second branches as the arterial third branches and ran parallel to them. The number and measurements of the branches are shown in Table 1.

Ductal branches
The main stem reached almost to the center of the gland while decreasing in diameter. An average of 4 to 6 first branches with an average diameter of 343.13 ± 74.02 μm diverged from the main stem like a tree along its length. These first branches diverged from the same point on their main stem as the arterial and venous first branches and ran parallel to them. Each first branch separated into 4 to 6 second branches with an average diameter of 192.62 ± 51.62 μm in a tree-like pattern along its length. These second branches diverged from the same location on their first branches as the arterial and venous second branches and ran parallel to them. Each second branch separated into 4 to 7 third branches with an average diameter of 107.30 ± 29.08 μm in a similar manner. These third branches diverged from the same location on their second branches as the arterial and venous third branches and ran parallel to them. The number and measurements of the branches are shown in Table 1.

Human submandibular gland (Figure 2)

Arterial branches
The main stem reached almost to the center of the gland while decreasing in diameter. An average 7 to 11 first branches with an average diameter of 2.44 ± 0.34 mm diverged from the main stem like a tree, staggered along its length. Each first branch separated into 7 to 11 second branches with an average diameter of 0.88 ± 0.34 mm, also in a tree-like pattern with staggered positions, and each second branch separated into 6 to 10 third branches with an average diameter of 0.32 ± 0.15 mm in a similar pattern. The number and measurements of the branches are shown in Table 2.

Venous branches
The main stem reached almost to the center of the gland while decreasing in diameter. An average of 7 to 12 first branches with an average diameter of 4.35 ± 0.89 mm diverged from the main stem like a tree, staggered along its length. These first branches di-
Fig. 1  Corrosion casts showing the branching aspects of the blood vessels and ducts in the rabbit submandibular gland.

Fig. 2  Corrosion casts showing the branching aspects of blood vessels and ducts in the human submandibular gland.
White: Ducts.
to 11 first branches with an average diameter of 1.27 ± 0.29 mm diverged from the main stem like a tree, staggered along its length. These first branches diverged from the same point on their main stem as the arterial and venous first branches and ran parallel to them. Each first branch separated into 6 to 9 second branches with an average diameter of 0.69 ± 0.34 mm in a tree-like pattern staggered along its length. These second branches diverged from the same location on their first branches as the arterial and venous second branches and ran parallel to them. Each second branch separated into 5 to 8 third branches with an average diameter of 0.27 ± 0.08 mm in a similar pattern. These third branches diverged from the same location on their second branches as the arterial and venous third branches and ran parallel to them. The number and measurements of the branches are shown in Table 2.

**DISCUSSION**

**Branching patterns in the submandibular gland**

The first, second and third branches of arteries, veins and ducts all diverged at the same points and ran parallel in both the rabbit and human submandibular glands. These findings show common branching characteristics between the two species. The location of divergence and the paths of the arterial, venous and ductal branches in the rabbit submandibular gland were thus extremely similar to those in humans.

**Comparisons of the number and diameter of each branch type in rabbit and human submandibular glands in the present study**

**Arterial branches**

The average number of first branches was 36% lower in the rabbit submandibular gland than in the human gland, 43% lower for the second branches, and 34% lower for the third branches. The average diameter of each first branch was 89% lower in rabbit glands than in that of humans, 80% lower for each second branch, and 72% lower for each third branch (Tables 1 and 2).

**Venous branches**

The average number of first branches was 41%
lower in the rabbit submandibular gland than in that of humans, 53% lower for the second branches, and 52% lower for the third branches. The average diameter of each first branch was 90% lower in rabbit glands than in that of humans, 86% lower for each second branch, and 78% lower for each third branch (Tables 1 and 2).

Ductal branches
The average number of first branches was 44% lower in rabbit submandibular glands than in that of humans, 43% lower for second branches, and 12% lower for third branches. The average diameter of each first branch was 75% lower in rabbits than in humans, 72% lower for each second branch, and 59% lower for each third branch (Tables 1 and 2).

The number and thickness of each artery, vein and duct branch type were lower in rabbit submandibular glands than in those of humans. The difference could be due to the smaller size of the rabbit gland, which is 15.80 ± 1.55 mm long and 9.15 ± 0.75 mm wide, compared to the human gland, which is 43.50 ± 1.10 mm long and 18.50 ± 0.60 mm wide, based on measurements by Xu et al.^

Comparison of our data with previous studies on the human gland
Xu et al.^
 counted the number of each branch type and measured the diameters of arteries and ducts in the human submandibular gland. They reported no significant difference between artery and duct branching, and their measurements produced results almost identical to this study. Xu et al.^
 did not measure veins, however, so those results could not be compared. Instead, the thickness of each venous branch type was compared to each matching arterial branch type based on our measurements. The diameter of each venous first branch was 78% greater than that of the arterial first branch, 50% greater for each venous second branch compared to its arterial counterpart, and 44% greater for each venous third branch compared to its arterial counterpart.

Partial autologous microvascular transfer of submandibular gland
Partial autologous microvascular submandibular gland transfer is a promising new treatment method for severe cases of keratoconjunctivitis sicca. Traditional autologous microvascular transfer involves transplantation of a submandibular gland to the lateral side of the eye fissure and insertion of Wharton's duct into the upper conjunctival fornix to replace tears with saliva secreted from the transferred gland. While this operation remarkably alleviated the disease, some patients became epiphora as a complication. Xu et al.^
 speculated that a reduction in the size of the transplanted gland could prevent postoperative epiphora. Because the effectiveness of a partial autologous microvascular submandibular gland transfer has not been confirmed, this new technique should first be examined with animal models.

The results of this study show that arterial, venous and ductal branching in the submandibular gland are similar between humans and rabbits. Therefore, we believe the rabbit is an appropriate animal model for investigating partial autologous microvascular submandibular gland transfer.

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