Doctor of Philosophy

The Rat Eustachian Tube: Anatomical, Histological and Radiological Findings

The graduate School

of the University of Ulsan

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The Rat Eustachian Tube: Anatomical, Histological and Radiological Findings

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A Dissertation

Submitted to

The Graduate School of the University of Ulsan

in partial Fulfillment of the Requirements for The Degree of

Doctor of Philosophy

by

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Department of Medicine

Ulsan, Korea

2019

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ACKNOWLEDGEMENT

I would first like to extend my sincere gratitude to my thesis advisor Prof. Song Ho-Young. Prof. Song gave me guiding proposal and recommendations on the direction of my thesis. He consistently allowed this paper to be my own work, but always offered key recommendations when I ran into a trouble spot or had some questions about my research work or writing scientific papers.

I am also deeply indebted to the experts who were involved in this research project: Prof. Park Hong Ju, Prof. Kang Woo Seok, and Prof Son Woo-Chan. Without their passionate participation and contribution, the project could not have been successfully conducted.

I would also like to thank Prof. Jeon Jae Yong and Prof. Kim Kun Yung as the second reader of my thesis, I really appreciate your valuable comments on this thesis.

Finally, I must express my gratitude to my beloved parents who have always been helping me out of difficulties and supporting without a word of complaint, and to my dedicated colleagues, Dr. Park Jung-Hong, Dr. Hu Hong Tao, Dr. Bakheet Nader, Mr. Yoon Sung-hwan, Mr. Ryu Dae-sung, Mr. Kang Jeon Min and Ms. Kim Jeong Seon, for their continuous support and encourage throughout my years of study and research in Korea. This

accomplishment would not have been possible without your support. Thank you.

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ABSTRACT

Background and purpose: Fluoroscopic Eustachian tube balloon dilation has been considered as an effective treatment for Eustachian tube dysfunction. However, many questions regarding the mechanism, recurrence and safety issues, and histological changes remains unclear. We hypothesized that a rat E-tube model could help to solve those questions. The purpose of our study was to investigate the anatomy, histology and radiology of the rat E-tube.

Materials and methods: A total of 15 male Wistars rats were used in this study: 5 for anatomical study, 5 for histological analysis, and 5 for tubography. Both ears of each rat were used in our study, so a total of 10 E-tubes were available for each study. Five rats were sacrificed, decapitated and micro-dissected to describe the anatomy of the E-tube. The full length, length of the bony portion, diameter of the tympanic orifice and other parameters of the E-tube were measured. Another five rats were sacrificed, and the coronary and longitudinal sections of E-tube were obtained to study the histology of E-tube. For the remaining five rats, the Eustachian tubography was carried out using a new trans-tympanic membrane (trans-TM) approach. The procedure time was measured from the insertion of the guidewire introducer to the removal of the micro-catheter after injection of contrast medium.

Results: The rat E-tube consisted of bony portion and membranous portion. The bony portion was covered by both cartilage and bone, while the membranous portion had no

coverage of the cartilage or bone. The diameter and the full length of the rat E-tube were 3.28 mm ± 0.26 mm and 4.96 mm ± 0.41mm, respectively. The diameter of the tympanic orifice was 1.18 mm ± 0.10mm. The E-tube was lined by pseudostratified ciliated epithelium with goblet cells. The tubography was successfully performed in all rats using a trans-TM approach. No procedure-related complications occurred. The mean procedure time was 4.7 minutes. From Eustachian tubograms, the E-tube, tympanic cavity and nasopharynx were clearly identified.

Conclusion: In the present study, we described the anatomical, histological and radiological findings of the rat E-tube. The tubography was performed successfully using a trans-TM approach. These findings may be valuable for the studies of the Eustachian tube dysfunction in the future.

Keywords: Eustachian Tube; Rat; Anatomy; Histology; Radiology; Eustachian tubography

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ETD	Eustachian tube dysfunction
E-tube	Eustachian tube
TC	tympanic cavity
TM	tympanic membrane
NP	nasopharynx
EAC	external auditory canal
LVPM	levator veli palatini muscle
TVPM	tensor veli palatini muscle
SPM	salpingopharyngeus muscle
BP	basisphenoid bone
TP	tympanic bone process
SMG	seromucous glands

INTRODUCTION

Eustachian tube (E-tube) dysfunction (ETD) is a common clinical diagnosis in otology practice, especially for the children younger than 7 years old[1]. A previous study has reported that the incidence of ETD was 0.9% in adult population and rising up to 70% in patients who underwent surgery for recurrent otitis media[2]. ETD may occur in the conditions of the swelling mucosa of the tube, or inability of E-tube to open[3]. Various approaches, such as topical nasal steroids, auto pressure equalization technique, treatment with decongestants, and antihistamines have been used to relief the symptoms[4,5]. Surgical procedures including ventilation tube insertion, laser tuboplasty, and microdebrider tuboplasty are usually indicated when the medical management fails[6-9]. However, the efficacy of these approaches remains uncertain due to differential diagnosis criteria and limited clinical experience.

Since Ockermann and colleagues[10] reported their first experience with endoscopic Eustachian tube balloon dilation (ETBD) in 2010, the procedure has been commonly used for the treatment of obstructive ETD[11-20]. However, the entire balloon catheter cannot be visualized during endoscopic ETBD[10,21,22]. In addition, despite the problem of false passages created by balloon catheters in endoscopic ETBD[21,23,24], using a guide wire has traditionally been considered contraindicated because of potential damage to the ossicles, tympanic membrane (TM), or inner ear[16,22].

Recently, fluoroscopy has been used for diagnostic or interventional procedures on the E-tube[22,25-28]. Kim et al.[22] reported that fluoroscopic ETBD using a flexible guide wire can offer the following advantages over conventional endoscopic ETBD: (a) general anesthesia is not needed; (b) false passage creation is avoided; (c) the location and inflation status of the balloon catheter in the ET can be visualized during the procedure. The clinical success rate of fluoroscopic ETBD was 78.1%, and its recurrence rate during the median follow-up of 15.9 months was 16%[28]. However, to our knowledge, no studies have investigated the diameter and radiological evaluations of the E-tube in rats. Therefore, many questions such as the mechanisms of fluoroscopic interventional procedures and recurrence, safety issue of using contrast medium in the E-tube, and histological changes after fluoroscopic interventional procedures remain to be answered. We, therefore, hypothesized that a rat E-tube model could help to solve these questions. The purpose of this study was to investigate the size and radiological findings of the rat E-tube, which can facilitate fluoroscopic E-tube interventions.

MATERIALS AND METHODS

Animals and study design

Fifteen male Wistars rats with the age of 16 weeks were used in this study. They were divided into 3 groups. Five rats in each group were used for the anatomical study, histological analysis, and Eustachian tubography, respectively. Both ears of each rat were used in this study, so a total of 10 E-tubes were available for each study. This study was approved by the committee for animal research at our institution and conformed to the US National Institutes of Health Guidelines on the care and use of laboratory animals.

Anatomical study

Five rats were euthanized using carbon dioxide asphyxiation, and decapitated immediately after death. The mandible, temporal bone, masseter, pterygoid, and the neck muscles were removed to expose the anatomical structures of the skull base. The E-tube and tympanic bulla (Fig. 1) were meticulously dissected from the ventral side to the dorsal side under a surgical microscope with a high magnification field.

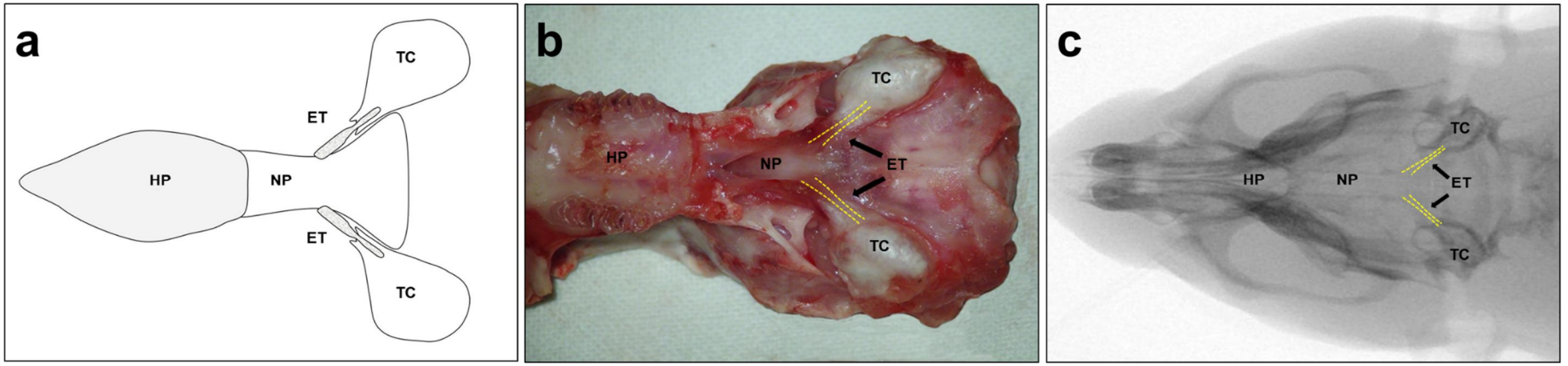

Fig. 1. Schematic (a), micro-dissection (b) and radiographic images (c) show the rat E-tube. HP: hard palate; NP: nasopharynx; TC: tympanic cavity; ET: Eustachian tube.

The photos of the nasopharynx, E-tube, both orifices of the E-tube, and the tympanic cavity were taken under the surgical microscope. The following measurements were obtained using Image J software (U. S. National Institutes of Health, Bethesda, State of Maryland, U.S): the full length of E-tube (Fig. 2a), the length of bony portion (Fig. 2a), the length of nasopharyngeal opening (slit-like shape) (Fig. 2b), and the diameter of tympanic opening (round-like shape) (Fig. 2c).

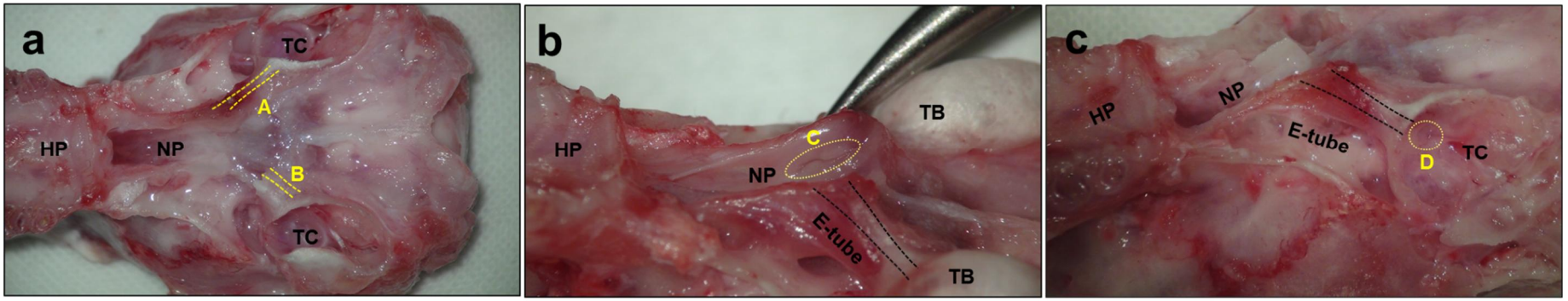

Fig. 2 The micro-dissection images show the rat E-tube (a), nasopharyngeal opening (b) and tympanic orifice (c). In Figure a, the right membranous portion of the E-tube and attached muscles were removed to show the bony portion of the E-tube. The following parameters were measured: A: full length of E-tube; B: length of bony portion; C: Length of nasopharyngeal opening (slit-like); D: Diameter of the tympanic orifice (round-like). HP: hard palate; NP: nasopharynx; TC: tympanic cavity; TB: tympanic bulla.

Histological analysis

Five rats were euthanized using carbon dioxide asphyxiation and decapitated, and then needless tissues were removed from the specimen. A tissue sample containing the E-tube, tympanic bulla, maxillary bone, and the skull was dissected and fixed in phosphate buffered formaldehyde (4%) for 48 hours. After decalcification in ethylenediaminetetraacetic acid (10%; pH 7.4), the specimen (n=10) was trimmed to an appropriate size, dehydrated in graded alcohol, and embedded in paraffin. The ET and adjacent tissues were serially sectioned in a coronary plane (n=6) and a longitudinal plane(n=4). Hematoxylin-eosin staining was performed in each section.

Eustachian tubography

Five rats were anesthetized with an intramuscular injection of 50 mg/kg zolazepam and tiletamine (Zoletil 50; Virbac, Carros, France) and 10 mg/kg xylazine (Rompun; Bayer HealthCare, Leverkusen, Germany). The procedure was performed under fluoroscopic guidance using a Metro R X-ray inspection system (NanoFocusRay Co. Ltd., Iksan, Republic of Korea). The rats were kept in a prone position during the procedure. A metal guidewire introducer (S&G Biotech, Yongin, Republic of Korea) with the inner diameter of 0.022 inches (Fig. 3) was introduced through the TM into the tympanic cavity, and was negotiated into the tympanic orifice of the E-tube (Fig. 4a). A 0.016-inch micro-guidewire (S&G Biotech, Yongin, Republic of Korea) was inserted through the introducer across the E-tube into the nasopharynx (Fig. 4b). And then the introducer was removed while the guide wire was in place (Fig. 4c). After that, a 2.1 Fr micro-catheter (S&G Biotech, Yongin, Republic of Korea) was advanced over the guidewire

into the tympanic opening of the E-tube (Fig. 4d). After removal of the guidewire out of the catheter (Fig. 4e), the contrast medium (Omnipaque 350, GE Healthcare Inc., Marlborough, U.S.) was injected through the catheter into the E-tube (Fig. 4f) in the rate of 120 ml/h using a syringe pump (KDS100, Kd Scientific, Holliston, MA, USA). During the injection, a series of Eustachian tubographic images was recorded. The diameter and full length of the E-tube, and the angle between the E-tube and sagittal plane were measured on the images using Image-J software (Fig. 5). The procedure time was measured from the insertion of the guidewire introducer to the removal of the micro-catheter after injection of contrast medium.

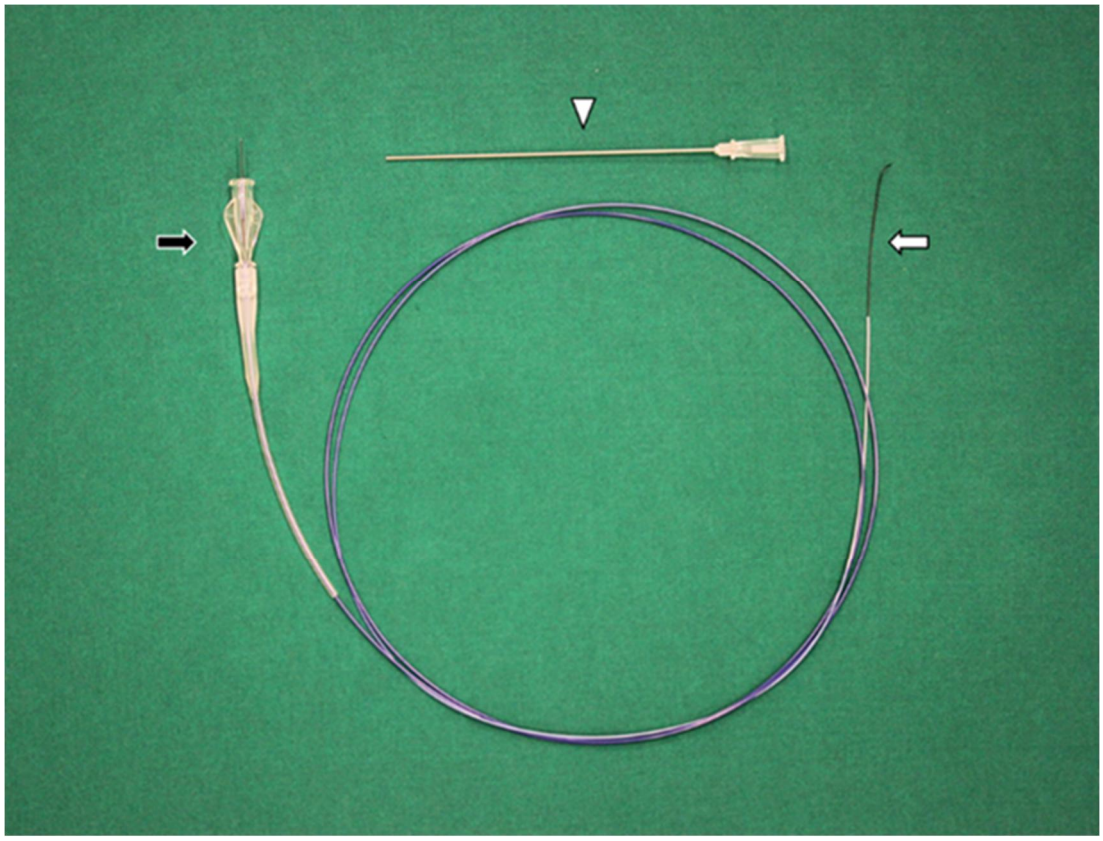

Fig. 3 The photograph shows the instruments for the Eustachian tubography, consisting of a 2.1 Fr. micro-catheter (black arrow), a 0.016-inch micro-guidewire (white arrow), and a 0.022-inch guidewire introducer (arrowhead).

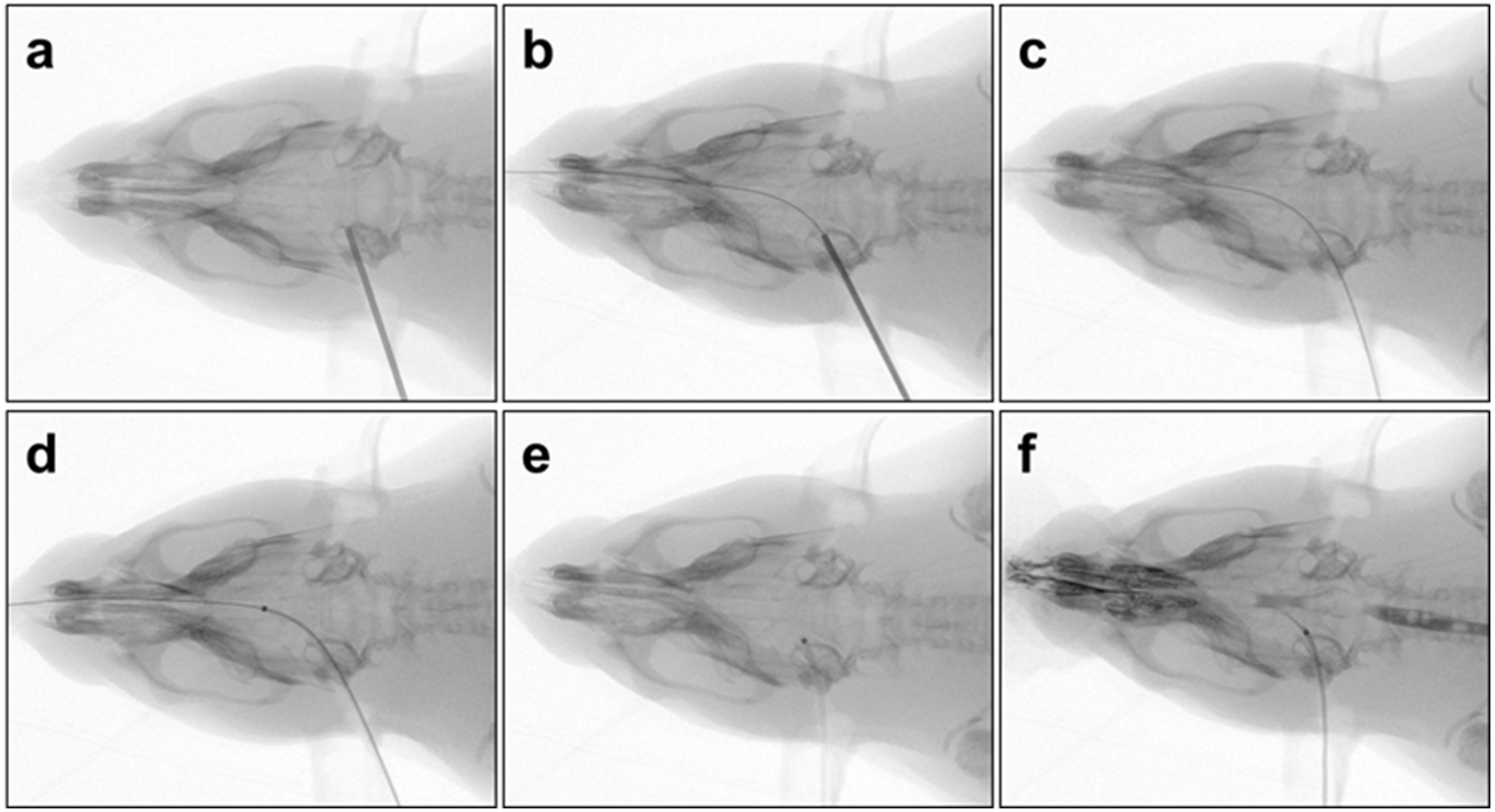

Fig. 4 Images show the procedures of the new trans-TM method to perform the tubography. (a) A metal guidewire introducer was introduced through the TM into the tympanic cavity, and was negotiated into the tympanic opening of the ET; (b) A micro-guidewire was inserted through the introducer across the E-tube into the nasopharynx; (c) The introducer was removed while the guide wire was in place; (d) A micro-catheter was advanced over the guidewire; (e) The tip of the micro-catheter was placed in the tympanic side of ET; (f) Tubography was performed by injecting contrast medium through the catheter.

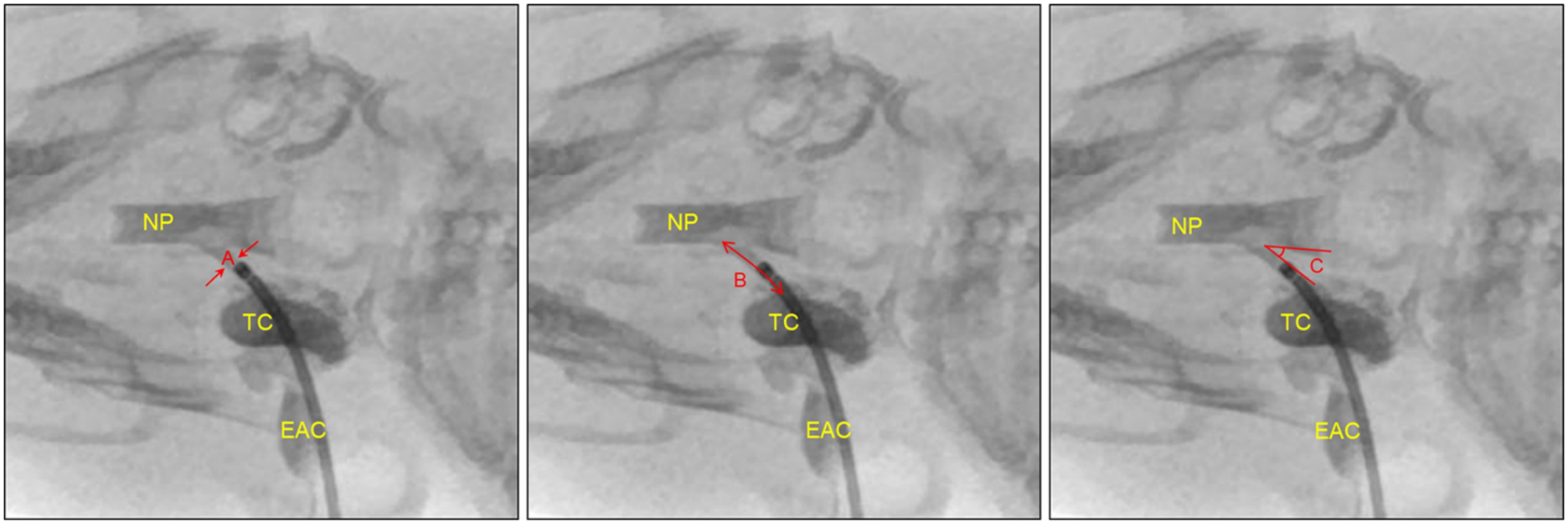

Fig. 5 Measurement of the rat E-tube in radiological images. A: Diameter of the E-tube; B: Full length of the E-tube; C: Angle between the E-tube and the sagittal plane.

Statistical analysis

Statistical analysis was performed using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA). The mean values and stand deviations of the full length of E-tube, length of bony portion, diameter of E-tube, angle between E-tube and sagittal plane, diameter of tympanic orifice, and the length of nasopharyngeal opening were calculated using the software.

RESULTS

Microdissection findings

The E-tube was straight and ran in a caudo-lateral direction from the nasopharynx to the middle ear cavity. It consisted of a bony portion and a membranous portion. The bony portion was longer than the membranous portion and occupied lateral 2/3 of the E-tube, which was covered by the bony and cartilaginous tissues. And its membranous portion occupied medial 1/3 of the E-tube and was not covered with bone or cartilage. The nasopharyngeal opening of the E-tube was encircled by two soft, lip-like, mucosal folds- one in the ventral side and one in the cranial side, both easily mobile. And its tympanic opening was round, and located in the rostro-medial wall of the tympanic cavity.

The sizes of selected parameters are shown in Table 1 by means of values and standard deviations.

Table 1 Measurement of the rat E-tube in micro-dissection images

Parameters	Mean	SD
Full length（mm）	4.96	0.41
Length of bony portion（mm）	3.28	0.26
Diameter of tympanic orifice（mm）	1.18	0.10
Length of nasopharyngeal opening（mm）	0.62	0.09

Histological findings

The E-tube was divided into the tympanic, middle and nasopharyngeal segments. Its opening at the tympanic segment was oval configuration in coronary section. A C-shaped cartilage was implanted between the mucosa and bone, and covered the whole mucosa except for a small area on the ventral side. The cartilage gradually decreased in size and then disappeared at the nasopharyngeal segment. The shape of the middle section was crescent. At the tympanic end of the middle segment, the ventral extension was completely covered by the medial process of the tympanic bone, but toward the nasopharynx, the size of the bony process gradually decreased and the ventral wall of the E-tube was supported by the levator veli palatini muscle (LVPM) and the salpingopharyngeus muscle (SPM). In the nasopharyngeal part, the tube almost ran in a horizontal direction. The tensor veli palatini muscle (TVPM), LVPM and SPM bordered the E-tube, which lacked any cartilage or bone covering (Fig. 6).

The E-tube was lined by pseudostratified ciliated epithelium with goblet cells. The number of ciliated cells and goblet cells in the nasopharyngeal segment was higher than that in the tympanic segment. More squamous cells were observed in the transitional area between the nasopharyngeal side of the E-tube and the nasopharynx (Fig. 7). The seromucous glands were mainly distributed under the submucosa layer in the dorsomedial area of the E-tube.

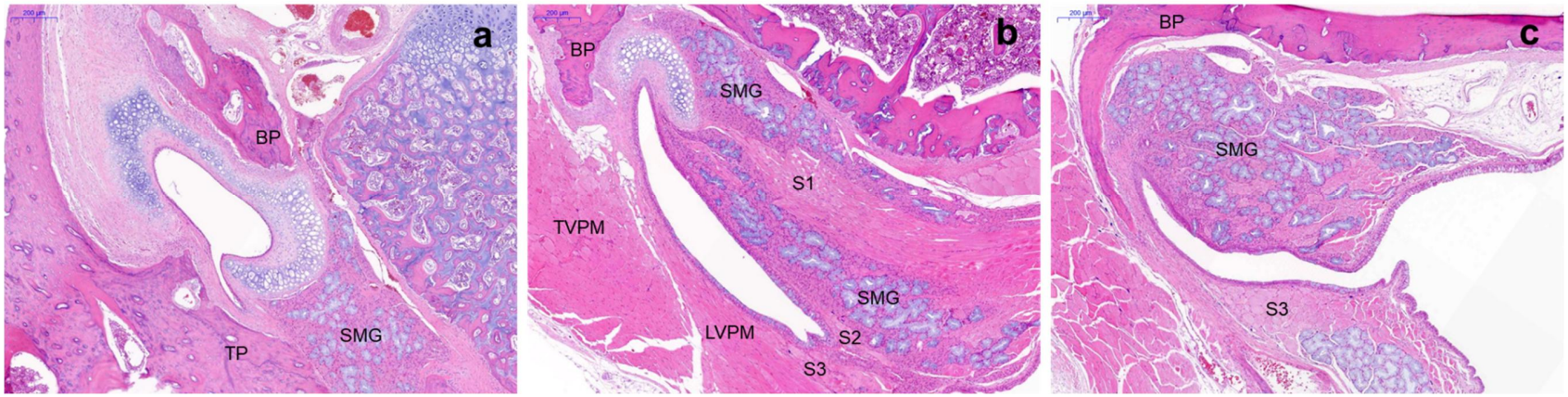

Fig. 6 Coronary sections of the rat E-tube from tympanic side to nasopharynx side showing the E-tube lumen and paratubal structures. (a) The tympanic segment; (b) The middle segment; (c) The nasopharyngeal segment. Bars: 200μm; BP: basisphenoid bone; TP: tympanic bone process; SMG: seromucous glands; TVPM: tensor veli palatini muscle; LVPM: levator veli palatini muscle; S1,S2,S3: subgroups of fibers of salpingopharyngeus muscle (SPM).

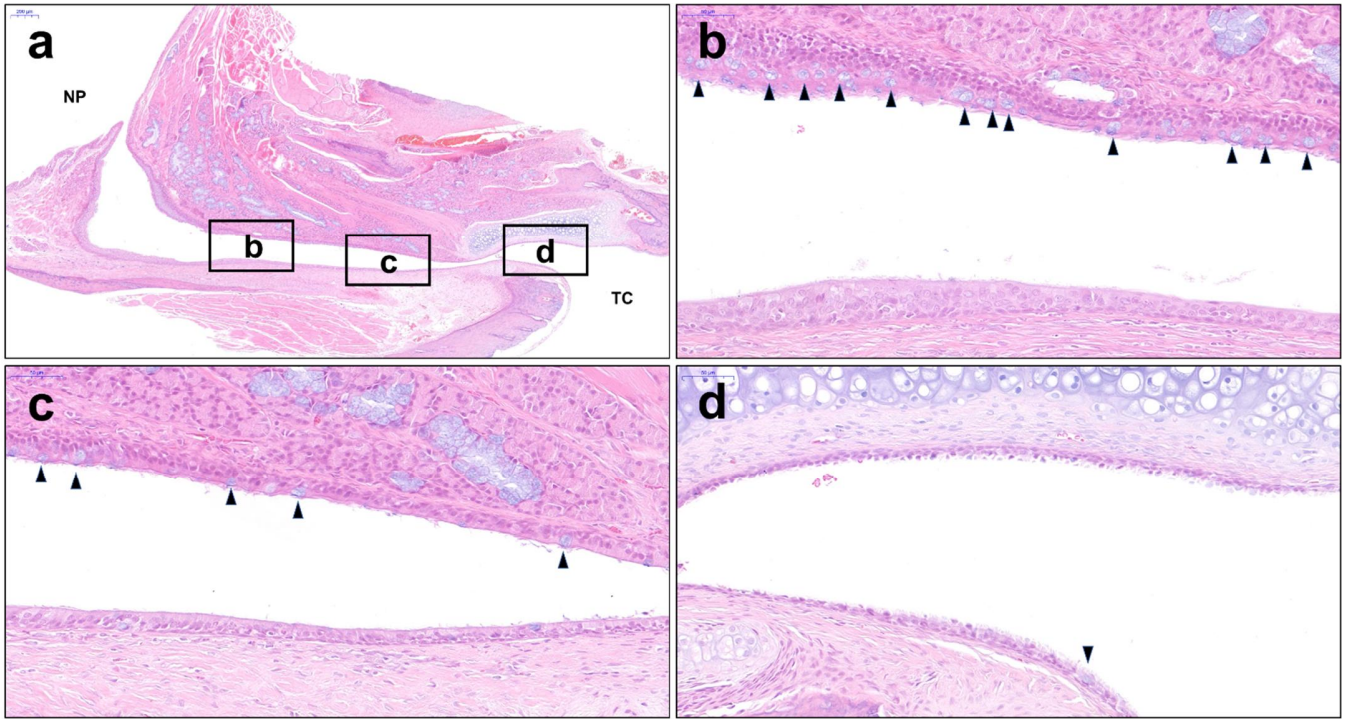

Fig. 7 The histological images of the rat E-tube by the ventro-dorsal longitudinal section. (a) An overview of the rat E-tube (4×); (b) Nasopharyngeal part of the E-tube (30×); (c) Middle part of the E-tube (30×); (d) Tympanic part of the E-tube (30×). Arrowheads indicate goblet cells. NP: nasopharynx; TC: tympanic cavity.

Radiological findings

The Eustachian tubography was successfully performed in all rats. The technical success rate of the procedure was 100%, and no procedure-related complications occurred. The mean time required for the procedure was 4.7minutes (range, 3.8-6.9 minutes) for each ear.

From the radiological images, the contour of the tympanic cavity was visualized due to the presence of the bony landmark (Fig. 1c). The medial process of the tympanic bone can be identified as a small piece of the high density protruding out of the rostro-medial wall of the tympanic cavity (Fig. 8).

From the Eustachian tubograms, the E-tube filled with contrast medium was identified. The nasopharynx also was seen after a small amount of contrast medium flowed into the nasopharynx (Fig. 5).

The diameter and full length of E-tube, and the angle between E-tube and sagittal plane were measured in the tubograms, and the values are shown in Table 2.

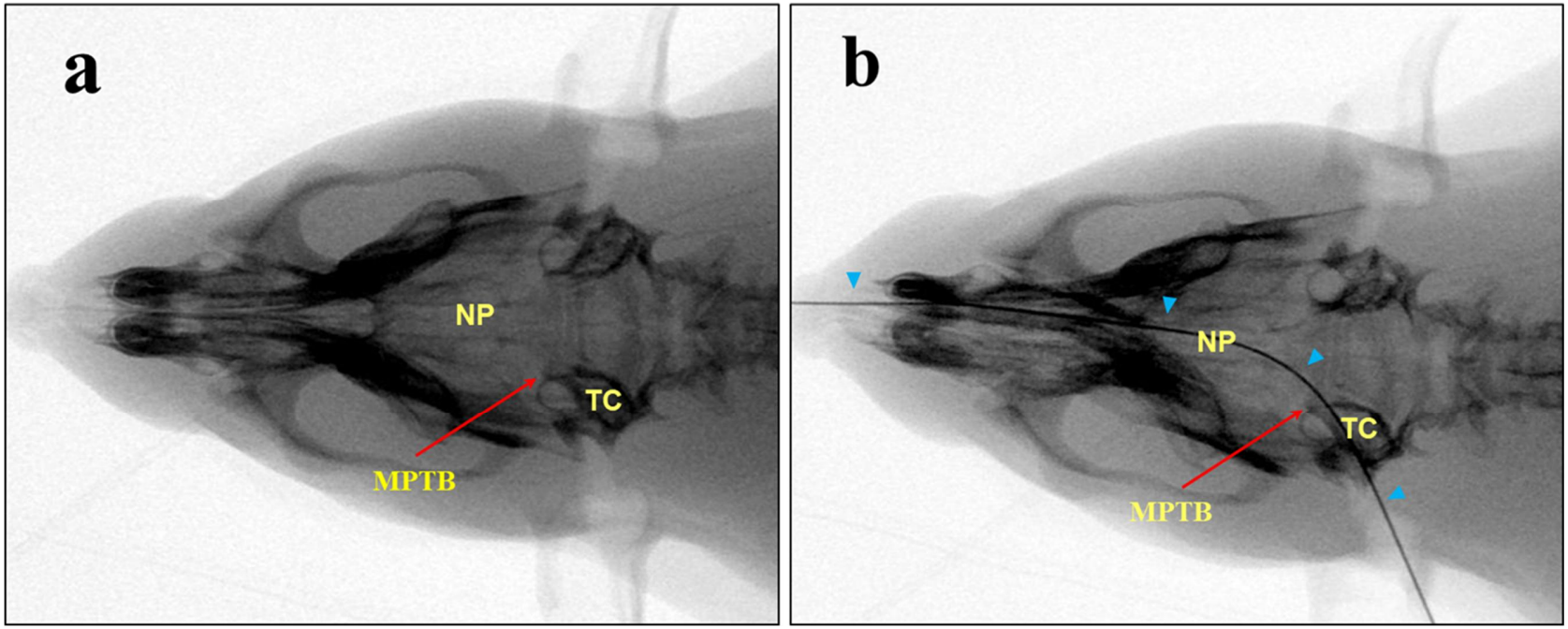

Fig. 8 Radiological images showed the medial process of tympanic bone. (a) No micro-guidewire passing through the E-tube; (b) A micro-guidewire (Arrowheads) passing through the E-tube to indicate the course of the E-tube. MPTB: medial process of tympanic bone; TC: tympanic cavity; NP: nasopharynx.

Table 2 Measurement of the rat ET in radiological images

Parameters	Mean	SD
Full length（mm）	4.60	0.29
Diameter（mm）	0.43	0.07
*Angle（degree）	34	3.81

*Angle: between E-tube and sagittal plane

DISCUSSION

This study reveals that the anatomical configration and histological analysis of the rat E-tube can accurately measure the length, diameter, and angles of the E-tube and that Eustachian tubography was simple, easy, and clearly showed the E-tube and its adjacent structures. On the basis of these observations, the rat E-tube can be a suitable model for facilitating fluoroscopic E-tube interventions in the treatment of ETD.

The rat E-tube is a narrow and intricate structure. Only a few studies have described the anatomical structures of the tube[29,30]. Most of the findings in the present study were consistent with previous studies. However, the purpose of this study was not only to observe anatomical structures of the tube but also to develop a new approach to perform the Eustachian tubography. Therefore, we gave more attention to the role of these findings in the feasibility of the rat Eustachian tubography. This study showed that the tympanic orifice of the E-tube located in the rostro-medial wall of tympanic cavity, opposite to the TM with a slight angle in the rostro-caudal direction. Also, there were no anatomical structures standing in the route between the tympanic orifice of the E-tube and the TM. The above findings suggest that we can negotiate the micro-guidewire into the tympanic orifice of the E-tube using the trans-TM approach. The entire E-tube was straight, which means the micro-guidewire and micro-catheter can pass through the tube into the nasopharynx. The histological images showed the LVPM, TVPM and SPM attached to the middle part and nasopharyngeal part of the E-tube. The contraction of the muscles caused the tube dynamically open and close. These two parts of the rat E-tube, which was analogous to the cartilaginous portion in human, have the functions of equalization of pressure across the TM and clearance of middle ear secretions. Therefore, it can be selected as the target site

of the interventional techniques, such as balloon dilation or stent placement, for the treatment of ETD in animal experiment.

The data of rat E-tube, such as the diameter and total length, and the length of the nasopharyngeal opening, have been reported in several previous studies[29-31]. However, the specific measurement methods for these data have not been described. Most of the data were only approximate values, and some data have not been included. In our study, we measured the parameters of the rat E-tube using Image J software. Also, we measured the diameter of the tympanic opening, which is important for choosing the suitable size of the guidewire introducer and micro-catheter, so that we can easily perform the tubography in the sequential experiment. To our knowledge, the diameter of the tympanic opening in rats has not been described in previous studies.

In the animal experiments, the trans-TM approach has been reported to inject the bacteria or endotoxins to induce the acute inflammatory reaction, or perform the surgical operation in the tympanic cavity. In the present study, a new method to perform the Eustachian tubography using trans-TM approach was developed. During the procedure, the guidewire introducer was easily inserted into the tympanic opening of the E-tube through the TM, and the micro-catheter was successfully placed in the tympanic side of the E-tube. The technical success of the procedure relied on the anatomical and histological knowledge regarding the rat E-tube. The procedure can be used in various aspects of ETD studies in the future. For instance, the ETD model can be created by injecting embolic agent through the micro-catheter placed in the E-tube. The model used to be created by the surgical method, which is complicated and time-consuming[32-34]. Also, we surmise that the balloon dilation for the rat E-tube using the same technique might be used to investigate

the therapy mechanism of balloon dilation on the ETD. In addition, the stent placement may be performed in rat E-tube to investigate the efficacy of the stent on the ETD. The medial process of the tympanic bone can be visualized under fluoroscopy guidance, which is a bony landmark to find the tympanic orifice because the tympanic portion of the E-tube covered by the medial process. It was very important to ensure that the introducer can successfully negotiate into the tympanic orifice of E-tube. In the subsequent Eustachian tubography, the E-tube, tympanic cavity and nasopharynx can be identified. These radiological findings are essential for the fluoroscopic E-tube interventions to treat the ETD.

There are some limitations in the present study. First, there are anatomical variations of the rat E-tube. The small sample size in our study could not reflect all variations, which might cause the limitation of the results to some extent. Even if the number of animals were increased, however, it was unlikely that all anatomical variations would be included due to the extremely low incidence of variations. Second, measurement error is inevitable because of the subjectivity of the measurement process. Nevertheless, our research implemented a precise measurement and yielded convinced outcomes compared to previous studies. Third, our study is mainly a descriptive study, which lacks the investigation of the functions of anatomical structures. This study focused on the feasibility of Eustachian tubography in a rat model based on anatomical findings. The further studies regarding the association between the function and anatomy of the rat E-tube need to be conducted.

In our present study, we described the anatomical, histological and radiological findings of the rat E-tube. The Eustachian tubography can be performed successfully using

a trans-TM approach. These findings could be valuable for the studies of the Eustachian tube dysfunction in the future.

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