MICRODISSECTION OF MINIATURE PIG EAR

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Abstract

Objective To investigate the suitability of miniature pigs as an animal model for otological research. Methods Microdissection of the temporal bone was performed on 10 miniature pigs and recorded on photographs. Results The morphology and measurement of the external, middle and inner ear and the lateral recess of the miniature pigs were obtained by microdissection. Conclusion Compared to traditional animal models, the miniature pig may be a better model for biomedical research because of its many similarities in physiological functions with humans. Similarities of the temporal bone structures, including the external, middle and inner ear and the lateral recess, between the miniature pig and human make the animal a potentially useful model for otological research.

Key words: Miniature pig; animal model; otological research.

Introduction

Animal models of human diseases have always played a central role in biomedical research for the exploration and development of new therapies \cite{1}. Crucial prerequisites for the development of safe preclinical protocols in biomedical research are suitable animal models that would allow for human-related validation of valuable research information gathered from experimentation with lower mammals \cite{1}. Since the development of the Minnesota miniature pig (or minipig) in 1949 at the Hormel Institute (USA) (England et al, 1954) \cite{2}, miniature pigs have been extensively used as a large animal model in many biomedical experiments (Polejaeva et al, 2000 \cite{3}; Screaton et al \cite{4}, 2003) and studies of artificial organs (Van Dorp et al, 1998; Xu et al, 2003). Among the reasons for their common use are their similarities in gross anatomy and physiology to humans, as well as economic advantages and ethical reasons.

In otologic research, such as middle ear implants, cochlear implant, stem cell transplant for sensorineural hearing loss (SNHL) and basic research on deafness, animal studies are an essential method of evaluation prior to clinical trials. New Zealand rabbits, guinea pigs and chinchillas are, among other small mammals, well established animal models, but their auditory system is significantly smaller compared to that of the human \cite{1}. In this paper, temporal bone microdissection in the miniature pig as a new animal model in otologic research was investigated.

Materials and methods

Ten miniature pigs of both genders, aged between 6 and 8 weeks, provided the material for this investigation. This kind of miniature pig (Fig. 1A) from the Chinese

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Agricultural University of Beijing for otologic research, called the Chinese experimental miniature pig, was derived from little swine from Guizhou Province, China, in 1985, and its genetic background is well understood. Its characteristics include inherent small size, early sexual maturity, rapid breeding, and ease of management (Yu et al, 2003) [8]. Its baseline biochemical and hematological parameters have been clearly established [9].

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Agricultural University of China (Permit Number: 27-2956). All surgeries were performed under general anesthesia with sodium pentobarbital, and maximum efforts were made to minimize suffering.

Microdissection of temporal bone

Anaesthesia and specimen preparation

Ten pigs were anaesthetized using ether inhalation. Nine animals were immediately sacrificed by exsanguinations from the axillary artery, and dissected within 12 h post mortem. The head was removed at the C2 level. Skull specimen was obtained in 1 animal through boiling without dissection.

Procedures were recorded on photographs using an Olympus DP72 camera attached to a ZEISS microscope and processed by the DP2SW software.

Exposure of the middle ear

Two approaches were adopted to expose the middle ear. One was through enlarging the posterior wall of the external ear canal (Approach 1), as in myringoplasty in humans, and the other through mastoidectomy (Approach 2). Approach 1 offered generous exposure of the middle ear. Approach 2 provided easy exposure of the cochlea.

Microdissection steps

A scalpel was used to cut soft tissue and a hack saw to cleave bony structures. Using a hand drill (Bien-Air Surgery SA, OSSEODOC, Switzerland) with flexible shaft and appropriate cutting burs, the cartilaginous and bony structures of the external auditory canal were removed until the tympanic membrane (TM) was exposed. Care was taken to avoid damage of the delicate structures of the TM and ossicular chain. After removing the TM, the ossicles were removed with tweezers and stored in a solution of 70% ethanol in purified water until further measurements. After the ossicles were removed, the cochlea, the vestibule and the semicircular canals were exposed. The auditory nerve was also exposed via the translabyrinthine route and so was the foramen of Lushka.

Results

Landmarks of the temporal bone and the tympanic membrane

Landmarks of the temporal bone

The pig skull appeared more compact with significantly smaller brain capacity compared to humans. Anatomically, the temporal bone was located in the same position as in humans including the squamous, mastoid and tympanic (which was not obvious) parts, as well as the styloid process. The mastoid air cell system lied medial to the temporomandibular joint and inferior to the tympanic cavity. The external ear canal was very long and orientated strictly upwards and backwards (Fig. 1B). The location of the mastoid and the external ear canal differed significantly from the human (Fig. 1B and C). The average length of the external ear canal was 26.32 mm and mean diameter was 4.48 mm. The styloid process was much longer and stronger than that in the human. The petrous bone housed the inner ear including the cochlea, vestibule and semicircular canal (Fig. 1D) and was similar with the human. The internal auditory canal (IAC) was short and the base of IAC had Bill’s bar (BB) and transverse bar (TB) as in humans that separated the nerves in IAC (Fig. 1E).

Fig. 1 The miniature pig and its temporal bone, tympanic membrane.

(A) The miniature pig. (B) The lateral view of temporal bone. (C) Medial view of temporal bone. (D) Lateral view of the petrous bone. (E) Medial view of the petrous bone. (F) The tympanic membrane (TM) in the opened meatus. (G) Coronal CT scan of a pig head showing a very long external ear canal (EEC) orientated strictly upwards and backwards. (H) Coronal CT scan of a pig head showing the mastoid air cell system (straight arrow head) medial to the temporomandibular joint and inferior to the tympanic cavity.
The tympanic membrane (TM) was seen directly in the opened meatus (Fig. 1F) and very similar to that of the human. It was slightly oval in shape and its dimensions were determined with the measurement tool included in the microscopy software. The maximum size was 5.27 mm, the minimum 4.97 mm (Table 1). Coronal CT scan of the pig head revealed the relations between the external ear canal, the mastoid and the middle ear (Fig. 1G, H).

**Middle ear**

**Tympanic cavity**

Removing the mastoid allowed easy exposure of the cochlea (Fig. 2A) and dissection from the external ear canal led to direct exposure of the middle ear (Fig. 2B). The pig tympanic cavity was similar to that seen in humans, measured approximately 6 mm by 7 mm. The greatest distance between the medial and lateral walls was 6 mm and the smallest 1 mm. The tympanic cavity had six walls as in humans. The roof, the anterior, medial and the lateral walls were similar to the human, but the floor and the posterior wall were not.

The roof was a relatively thin plate of bone separating the cavity from the middle cranial fossa. The anterior wall was divided into a lateral recess and a medial portion which contained the tympanic opening of auditory tube (TOAT) (Fig. 2B). The lateral wall was the TM as in the human, which was inclined at an angle of 25° with the floor of the external ear canal (Fig. 1F). The medial wall cavity separated the middle ear from the inner ear. The promontory as the most obvious feature of the medial wall was the bone overlying the basal turn of the cochlea (Fig. 2C, D). The round window (RW), oval window (OW) as well as the fiber of the tensor tympani muscle (TT) was all visible (Fig. 2B, C, D).

The posterior wall (Fig. 2B) was the bone of the fallopian canal, housing the facial nerve, not the mastoid and tympanic antrum as in humans. The floor of the cavity was the mastoid (Fig. 1B) which would be the posterior wall in humans. It had a honeycombed appearance and led into a system of air cells within the cavities. In contrast to the human, no obvious aditus or antrum was identified.

![Fig. 2 Views on a middle ear (ME).](image-url)

(A) The right middle ear after removal of the mastoid. The artificial mastoid cavity (MC) is not well pneumatized. (B) Contents of the tympanic cavity: the short incus process (SP), long incus process (LP) and the stapes (S) are visible. Caudal to the oval window (OW), separated by the chorda tympani (CT), the round window (RW) can be found. (C) As in humans, the stapes with its head (SH) is encircled by the facial nerve (FN) which delivers the tympani chord (CT) caudally. (D) The round window (RW) as well as the fiber of the tensor tympani muscle (TT) can be identified. On the promontory, the turns of the cochlea can be seen.

(MH) the malleolar head, (FC) front crura, (PC) posterior crura, (I) incus, (ISJ) incudostapedial joint, (JN) Jacobson’s nerve, (HM) handle of malleus.

### Table 1. Measurement of the ear and comparison between miniature pig, pig and human from literature including ossicular chain, TM, OW

<table>
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<tr>
<td>malleus (mm)</td>
<td>L: 5.01(±0.5) EL: 4.54(±1.5)</td>
<td>L: 4.8(±0.4) EL: 4.9(±1.4)</td>
<td>EL: 4.4-5.8</td>
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<td>incus (mm)</td>
<td>EL: 2.59(±0.16) W: 2.69(±0.15)</td>
<td>EL: 3.12(±0.15) W: 3.05(±0.14)</td>
<td>EL: 4.99-5.0 W: 4.36-7</td>
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<tr>
<td>stapes (mm)</td>
<td>H: 2.13(±0.15) W: 1.88(±0.15)</td>
<td>H: 2.16(±0.14) W: 2.05(±0.14)</td>
<td>H: 2.61-3.32 W: 2.30-3.03</td>
</tr>
<tr>
<td>stapes angle( )</td>
<td>A: 31(±3.0)</td>
<td>A: 32(±2.0)</td>
<td>A: 20</td>
</tr>
<tr>
<td>TM area(mm²)</td>
<td>30.49(±3.37)</td>
<td>44.49(±3.37)</td>
<td>50-88</td>
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<tr>
<td>TM semi-axis(mm)</td>
<td>SMa: 5.27(±1.47),SMi: 4.97(±1.35)</td>
<td>SMa: 11.6(±1.36),SMi: 9.86(±1.24)</td>
<td>SMa: 7.5-9 Mi: 7.5-9</td>
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<tr>
<td>OW area(mm²)</td>
<td>1.12(±0.14)</td>
<td>1.24(±0.13)</td>
<td>2.65-3.75</td>
</tr>
<tr>
<td>OW semi-axis(mm)</td>
<td>SMa: 0.9(±0.2) Mi: 0.9(±0.2)</td>
<td>SMa: 1.7(±0.1) Mi: 1.0(±0.1)</td>
<td>SMa: 2.3-3.0 SMi: 1.08-1.66</td>
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Contents of the tympanic cavity

Ossicular chain

After removal of the tympanic membrane, a morphologic buildup relatively similar to humans was seen, including the ossicles (Fig. 3A-D), muscles and nerves. The ossicular chain together with the tympanic membrane formed the transducer mechanism of the middle ear. The malleus was attached to the tympanic membrane along the length of its handle and formed the most lateral ossicle (Fig. 3A, B). The stapes closed the oval window, formed the most medial ossicle and its footplate, thus separating the middle ear cleft from the inner ear (Fig. 3A, C).

The malleus in the pig showed a typical constitution with a head, neck, and handle and possessing lateral, medial and anterior processes (Fig. 3A, B). The head was ovoid in shape and lay medial to the scutum. The articular surface of the head was saddle shaped, covered with cartilage and formed a synovial joint with the corresponding articular face of the incus. Below the malleolar head the bone attenuated before giving rise to 3 processes as well as to the manubrium or handle and the medial process received the insertion of the tendon of tensor tympani that took the same course through the middle ear, as it does in humans. The incus was slightly more compact and smaller than the human having a body and 2 processes. Different than humans, both had the same average length of 1.8 mm and were perpendicular to each other (Fig. 3A, C), whereas in humans, they are a long and a short process. It articulated with the malleus via a saddle shaped articular surface (Fig. 3A, B). The stapes showed an identical morphology to that in the human. It consisted of a flattened head, 2 crura and an oval footplate (Fig. 3A, D). The head articulated with the incus and projected laterally. 3-D reconstruction of the ossicular chain was shown in Fig. 3E.

Measurement of the ear and comparison between miniature pig and human from literature including ossicular chain, TM, OW are shown in Fig.3F-J and Table 1.

Muscles and nerves

The origin of the tensor tympani muscle was found in a shallow depression situated in the medial wall of the tympanic cavity, which extended anteriorly into a conical bony pit as the cochleariform process (Fig. 1D) as in human from which the muscle fibers took their origin (Fig. 2D). From their broad origin the muscle fibers converged and then passed laterally at an angle of 90° to insert into the medial process of the malleus. The stapedius muscle was seen to arise from a depression in the posterior wall of the tympanic cavity deep to the overhang of the facial nerve canal. The muscle was attached to the lateral part of the posterior crus of the stapes by a delicate slender tendon (Fig. 2C).

The nerves followed a similar course to that of the human. The facial nerve was anatomically similar to the human one with a turn at the tensor tympani muscle and formed the first genu (Fig. 2D). The facial nerve showed an anatomical relation to the lateral arcade and formed the second genua (Fig. 2B) above the oval window as in humans. The facial nerve ran in an anteroposterior direction in the fallopian canal between the first and second genu (Fig. 2D), then passed inferiorly to exit from the canal through the stylomastoid foramen situated between the mastoid process anteriorly and the styloid process posteriorly, which are reversed in humans. The chorda tympani nerve branched from the facial nerve and ran in the temporal bone on the posterior wall of the tympanic cavity before entering the cavity itself and then passing posteroanteriorly between the long process of the incus and the malleus above the attachment of tensor tympani (Fig. 2B). The tympanic nerve formed a plexus beneath the mucosa of the promontory, grooving the bone (Fig. 2B, D).

Discussion

Pigs and miniature pigs as animal models for medical science studies

Animal models of human diseases have always played a central role in biomedical research for the exploration
and development of new therapies\textsuperscript{[1]}. However, the evolutionary gap between humans and many of the applied animal models (such as rodents) has always hampered a direct applicability of the knowledge gained for human therapy\textsuperscript{[1]}. Crucial prerequisites for the development of safe preclinical protocols in biomedical research are suitable animal models that would allow for human-related validation of valuable research information gathered from experimentation with lower mammals\textsuperscript{[1]}. Thus a suitable animal model is very important in biomedical research. The rodents and non-human primates (for ethical reasons) are not always the suitable animal models.

Being a domesticated eutherian (placental) mammal, the pig has evolved similarly with humans and represents a taxon with diverse selected phenotypes and the pig also represents an evolutionary clade distinct enough from primates and rodents to provide considerable power in the understanding of genetic complexity. The similarities between numerous physiological functions of pigs and humans have stimulated a wide range of biomedical research, such as reproduction, transgenesis, epithelial, and neural stem cell research, in which porcine pigs tend to be large in size and difficult to manage. Different types of miniature pigs have been bred including the Chinese experimental miniature pig\textsuperscript{[9]} (Fig. 1A). Its characteristics include inherent small size, early sexual maturity, rapid breeding, and ease of management (Yu et al, 2003). Compared to the rodent, the advantage is obviously - the miniature pig is more similar to the human, the result of the biomedical research with it is more valid. Compared to the non-human primates, there is less ethical concerns with pigs. Compared to traditional pigs, the miniature pig makes more economical sense, besides its other characteristics mentioned above. Miniature pigs have been extensively used as a large animal model in many biomedical experiments and studies of artificial organs\textsuperscript{[11,14]}

With otologic research, such as middle ear implant, cochlear implant, stem cell transplantation, gene therapy, pharmacology clinical trials and mechanisms of deafness, as well as with ear surgery training, the miniature pig can also be a suitable animal model.

**Measurement of middle ear and Miniature pigs in teaching ear surgeries**

On the outside, the miniature pig temporal bone appears completely different from the human, especially regarding the position of the external ear canal and mastoid. Its middle ear, however, is similar to that of the human and allows application of the miniature pig in otological research. Four of the six walls of the pig tympanic cavity are similar to those of human. The contents of the tympanic cavity including the ossicular chain, the muscles and nerves are also similar to the human.

In the absence of established methods of measurement for porcine ossicles, those common for human ones were used as far as available and reasonable. Determined were length of the manubrium according to Nomura et al.\textsuperscript{[13]} and the effective lever, assuming the center of ratio during oscillation to be in the head (Fig. 3F). Thus, for the porcine malleus, a circle was constructed, matching its head. The distance of its centre to the umbo along the axis of the manubrium was taken as the effective lever. Height and width of the incus and its effective lever were determined according to Nomura et al.\textsuperscript{[13]} (Fig. 3H). Dimensions of the stapes were determined according to Farahani and Nooranipour\textsuperscript{[16]}, namely height and width as well as the angle between both crura (Fig. 3G). The dimensions of the ossicles determined are comparable to the values determined by Pracy et al.\textsuperscript{[7]}. Measurement of the ear and comparision between miniature pig, pig and human from literature including ossicular chain, TM, OW were shown in Fig.3F-J and Table 1. The dimensions of the ossicular bones are smaller than the pig and human from literature in this study especially of the incus, TM semi-axis and TM area, despite previous reports\textsuperscript{[7, 18]} showing that the dimensions are similar to the human. The malleus, stapes, OW semi-axis (Fig. 3I) and OW area are are similar to the pig and human from literature. The difference between pig and miniature pig may be due to different breeds of being used for investigations.

The similarities between human ear and that of the miniature pig make the latter suitable for ear surgery training\textsuperscript{[19]}. In this study, one of the two approaches adopted to expose the middle ear is via expansion of the posterior wall of the external ear canal, as in myringoplasty in humans. By this approach, major surgical procedures including myringoplasty, ossicular chain reconstruction with prostheses or incus interposition, as well as CI and stapes procedures, can be practiced. Its long and narrow external ear meatus may be a good solution for practicing meatoplasty as described in ENT literature\textsuperscript{[19]}. For beginners in ear surgery, practicing bi-manual operation with suction in one hand and a microsurgical instrument in the other can be done with animal models as they provide the same physical behaviors as humans. Basic techniques as drilling and milling can be learned before starting anatomical training with human temporal bones.

**Conclusion**

Compared to traditional animal models, the miniature pig has several advantages for biomedical research including its similarities of numerous physiological functions with human. Its similarities to the human regarding the temporal bone include the middle and inner ear and the lateral recess and provide opportunities for its appli-
cation in otological research.

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References


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