Video Article

A Surgical Procedure for the Administration of Drugs to the Inner Ear in a Non-Human Primate Common Marmoset (*Callithrix jacchus*)

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Abstract

Hearing research has long been facilitated by rodent models, although in some diseases, human symptoms cannot be recapitulated. The common marmoset (*Callithrix jacchus*) is a small, easy-to-handle New World monkey which has a similar anatomy of the temporal bone, including the middle ear ossicular chains and inner ear to humans, than in comparison with that of rodents. Here, we report a reproducible, safe, and rational surgical approach to the cochlear round window niche for the drug delivery to the inner ear of the common marmoset. We adopted posterior tympanotomy, a procedure used clinically in human surgery, to avoid manipulation of the tympanic membrane that may cause conductive hearing loss. This surgical procedure did not lead to any significant hearing loss. This approach was possible due to the large bulla structure of the common marmoset, although the lateral semicircular canal and vertical portion of the facial nerve should be carefully considered. This surgical method allows us to perform the safe and accurate administration of drugs without hearing loss, which is of great importance in obtaining pre-clinical proof of concept for translational research.

Video Link

The video component of this article can be found at <https://www.jove.com/video/56574/>

Introduction

Sensorineural hearing loss (SNHL) arises predominantly from damage or deficiency in the cochlea. Common causes of SNHL include aging (e.g. presbycusis), genetic defects, exposure to loud noise, infection, and ototoxic drugs¹. The World Health Organization (WHO) estimated that over 360 million people, representing 5.3% of the world's population, suffer from hearing loss². It is also estimated that 1 in 900 to 1 in 2,500 newborns have moderate, severe, and profound congenital permanent hearing loss, and around one in three adults older than 65 years have some degree of hearing loss³. However, there is no effective clinical treatment of hearing loss for these patients.

Hearing research has long been performed using rodent or guinea pig models, and many approaches, such as gene therapy and regenerative therapy, have been suggested as a new treatment for hearing loss. However, there is a large difference between humans and rodents in terms of the auditory system, and it is very difficult to translate animal models to human applications. The common marmoset (*C. jacchus*), a New World monkey originating from the Amazon, has become an attractive non-human primate model for various basic research studies for several reasons. First, its anatomy and physiology are more similar to those of the humans rather than rodents. Second, the basic biological information about this species, including diseases, neural networks, behavior, and the genome, is well characterized. Auditory and vocal processing, the cortical coding of periodicity, the representation of pitch, and the auditory-vocal interactions of common marmosets have also been reported^{4,5,6,7,8,9,10}. In recent histological studies, researchers identified distinctive expression patterns of 20 deafness genes and anion exchangers in the cochlea of the common marmoset and found that five genes that are causative of progressive deafness, and three anion exchangers, had different expression patterns from those of rodents^{11,12}. These profiles lead us to believe that the common marmoset is a powerful tool for hearing research.

The most marked attributes of the common marmoset as an experimental animal are as follows:

- Easy handling with a small body size compared to some species of Old World monkeys: Adult marmosets weigh 300 - 400 g and are approximately 60 cm in height, which is similar to rats.
- Highly reproductive primate: Marmosets attain sexual maturity at an age of 18 months and are able to bear twice a year and produce 4 to 6 offspring annually.

- Genetic modifications are available: Sasaki E *et al.* succeeded in creating transgenic¹³ and knock-out¹⁴ primates using marmosets by genetic modifications implicated in neurological disorders (e.g., Parkinson's disease and Alzheimer's disease).
- Both embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have been established^{15,16}. Though it is rather difficult to keep the same number of marmosets compared to rodents, the availability of stem cells or iPS cells provides *in vitro* assays which will reduce the number of *in vivo* assays required.

To facilitate better translational research in the field of deafness and its potential therapy, we established an imaging study protocol using CT and MRI, general anesthesia and hearing tests using auditory brain stem responses (ABRs). These experimental systems may provide us with better opportunities to obtain pre-clinical proof of concept studies which are essential for bridging the gaps between rodent studies and clinical trials. Here, we report a surgical method to administrate drugs to the inner ear of the common marmoset through the round window membrane. To obtain a clear view around the round window, without manipulating the tympanic membrane which might cause a conductive hearing loss, it is useful to approach from the mastoid cavity. Clinically, this approach, referred to as "posterior tympanotomy" is well established and usually used for cochlear implantation and cholesteatoma surgery. We believe that posterior tympanotomy allows us to perform the accurate administration of drugs without inducing hearing loss.

Protocol

All experimental procedures were performed at the Jikei University, Tokyo Japan. Animal handling and experimental procedures were approved by The Institutional Animal Care and Use Committee of The Jikei University (Approval no: 26-060) and performed in accordance with the Institutional guidelines for use of laboratory animals at the Jikei University, which agree with the Guidelines for Proper Conduct of Animal Experiments by the Science Council of Japan (2006). Animal care was conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

1. Preoperative Examination

- Take a cranial Computed Tomography (CT) scan of animals (common marmosets) in order to facilitate the identification of individual anatomical structures at least one month before surgery.
- Carry out an audiometric test at least one month before surgery.
NOTE: Here, we used the ABR to assess hearing status. Briefly, a positive electrode was subcutaneously placed at the vertex and a reference negative electrode at the dorsal base of the stimulated ear. The ground electrode was subcutaneously placed at the dorsal part of the neck.^{17,18} ABRs were evoked with tone burst (1.0 ms tone burst duration with 0.1 ms rise and fall time). The sound level was then reduced in 10 dB steps from its maximum amplitude. At each sound level, 512 responses were averaged (with stimulus polarity alternated). ABR threshold was defined as the lowest sound pressure level (SPL) at which any wave could be detected.

2. Anesthesia

- Fast animals from the night before surgery under general anesthesia.
- Administer 4% isoflurane and ambient air at 5 - 6 mL/min with mask-to-face ventilation or in an induction chamber for slow induction (**Figure 1A**). Confirm that the animal does not respond to painful stimuli, such as the toe pinch.
- Anesthetize common marmosets with intramuscular injection (into the femoral muscle) of a combination of medetomidine (0.04 mg/kg), midazolam (0.4 mg/kg), and butorphanol (0.4 mg/kg).
- Inject ampicillin (0.08 mg/g) subcutaneously 30 min or more prior to skin incision as a preoperative antibiotic.
- Place the marmoset in the supine position with its head toward the top, and extend the head and neck. Fix the maxilla to the table with a nylon thread, and pull up the tongue to obtain a good view, or use a laryngoscope to visualize the epiglottis and the entry of the larynx alternatively (**Figure 1B**).
- Insert an endotracheal tube along the laryngeal surface of the epiglottis.
NOTE: It is possible to use a 16-gauge intravenous catheter, or 6-French gauge feeding tube, as an endotracheal tube (**Figure 1C**).
- Check the EtCO₂ from the endotracheal tube in order to confirm that it has been properly placed into the trachea. After the tube has been positioned and respiratory gas movement has been checked, fix the endotracheal tube to the marmoset's face with tape.
- Maintain anesthesia between 1-3% Isoflurane during the operation.
- Monitor the SpO₂, EtCO₂, body temperature and rectal temperature during operations. Perform all procedures on a heating mat to prevent a reduction in body temperature.

3. Preoperative Preparation

- Prepare the marmoset in a supine position, and incline the head to a 30 - 45 degree angle to the non-operative side (**Figure 2A**).
- Shave the post-auricular region of the operative side.
- Disinfect the surgical area with alcohol and povidone iodine, both in the post-auricular region and the external auditory canal.
- Place a surgical drape sheet with a round hole onto the marmoset (**Figure 2B**).

4. Surgical Procedures

- Inject 1% of lidocaine hydrochloride (with 1:100,000 epinephrine, 0.1-0.2 mL) subcutaneously into the skin incision site. Make an incision in the postauricular skin with a No.15 round-shaped blade.
- For hemostasis, apply pressure to the bleeding area using a gauze soaked in 1:5,000 diluted epinephrine. Bipolar cauterization is also effective (**Figure 2C**).

3. Open up the soft tissue of the post-auricular region so that auricular cartilage (c) and post-auricular muscle (m) can be recognized. Expose the external auditory canal bone that should be visible deep in the cartilage (**Figure 3A**).
4. To confirm the depth of the tympanic membrane, expose the bony part of the tympanic rim which can be recognized as the adhesion of skin and bone in the deepest part of the posterior ear canal.
NOTE: This step allows us to recognize how deep the lateral part of the middle ear cavity is (**Figure 3A**).
5. Peel the post-auricular muscles (sternocleidomastoid muscle, splenius capitis muscle, and posterior belly of digastric muscle) toward the caudal aspect, separated from the temporal bone. Then expose the lateral surface of the temporal bone (asterisk in **Figure 3B**).
6. Make a hole approximately 5 mm behind the external auditory canal with a 1.0 mm diamond bur. (**Figure 3C**). Use a drill speed of 1,000 - 8,000 rpm depending on the drilling site.
NOTE: A slow speed is adequate for delicate manipulation near the semicircular canal or the facial nerve. Drip the saline in drilling site properly to prevent the thermal injury.
7. Enlarge the hole by drilling and confirm the position of the horizontal semicircular canal (HSC), which bulges toward the mastoid cavity (**Figure 3D**).
8. Confirm the position of the facial nerve whose vertical part runs anterior of HSC. Drill and remove all the bone septa, if they exist, to obtain a clear view of the facial recess, which is located at the center of the triangular area between the facial nerve and the chorda tympani.
9. Make a small hole by drilling in between the vertical part of the facial nerve and the tympanic rim (asterisk in **Figure 4A**) with a 0.6 mm diameter diamond bur at 1,000 rpm.
10. Move the bur parallel to the posterior canal wall to prevent damage to the facial nerve, which can be seen as a pink line through the thinned bone. Stop the drilling immediately before the nerve is exposed as a white line structure (**Figure 4B**).
11. Ensure the round window niche can be visualized through the hole (**Figure 4C**). Then, administrate drugs into the niche with a 25-gauge or thinner needle. In order to retain drugs in the round window niche, place a viscous solution on the niche (**Figure 4D**).
12. After confirming that hemostasis is securely controlled, close the layers (epidermal layer and dermal layer) with simple interrupted suture using 6-0 absorbent threads.
13. Fix gauze on the surgical wound and affix a bandage.

5. Postoperative Care

1. Administrate atipamezole hydrochloride (0.15 mg/kg) intramuscularly into the femoral muscle to antagonize medetomidine.
2. When spontaneous breathing starts, remove the endotracheal tube.
3. Maintain the common marmoset in an intensive care unit at 30% O₂, 29 # until anesthesia wears off to complete arousal.
4. Confirm that there are no findings suggestive of vestibular dysfunction such as nystagmus, and return the animal to the breeding cage.

Representative Results

Posterior tympanotomy was performed without any complication such as surgical site infection, bleeding with vascular injury, or vestibular dysfunction, which sometimes occurs with middle ear manipulation. We performed posterior tympanotomy on the right ear of 7-year-old common marmoset and administered 1 μ L of phosphate buffered saline to the round window niche. ABRs were measured before and two months after surgery (**Figure 5A-B**). Waveforms and thresholds of ABRs were comparable to a previous study^{17,18}, and there was no change in these parameters before and after surgery (**Figure 5C**). Thus, we concluded that posterior tympanotomy is a safe and useful procedure for the administration of drugs to the round window niche of the common marmoset.

Figure 1: Tracheal intubation. (A) Anesthetize the common marmoset with 4% isoflurane and ambient air at 5-6 mL/min mask-to-face ventilation. Alternatively, place the common marmoset into an induction chamber. (B) Place the common marmoset in the supine position with its head toward the top, and extend the head and neck. Fix the maxilla with nylon thread to the table and pull up the tongue to visualize the epiglottis (arrowhead) and the entry of the larynx. (C) Insert an endotracheal tube along the laryngeal surface of the epiglottis. 16-gauge intravenous catheter or 6-French gauge feeding tube can be used as an endotracheal tube. [Please click here to view a larger version of this figure.](#)

Figure 2: Skin incision. (A) Place the common marmoset in the supine position, and incline the head to 30-45 degree angles to the non-operative side. Shave the operation side post-auricular region and disinfect with povidone iodine. Cr, cranial side, Ca, caudal side, L, left side, R, right side. (B) The black line indicates skin incision line. Before the skin incision, inject 1% lidocaine hydrochloride (with 1:100,000 epinephrine, 0.1-0.2 mL) subcutaneously. (C) Cut open subcutaneous tissue until the auricular cartilage (c) is elevated. [Please click here to view a larger version of this figure.](#)

Figure 3: Drilling into the mastoid cavity. (A) After removal of soft tissue, the post-auricular muscle will be visible. m, post-auricular muscle, c, auricular cartilage. (B) Temporal bone surface (asterisk) is exposed when the post-auricular muscle is peeled from the bone toward the caudal aspect. The pneumatized structure can be easily recognized through the temporal bone. (C) A hole was made by drilling with a 1.0 mm diamond bur approximately 5 mm behind the external auditory canal bone. (D) The mastoid cavity of the common marmoset is mostly composed of a single cavity. By enlarging the drilled hole, the horizontal semicircular canal (HSC and dotted line) bulges toward the mastoid cavity can be easily recognized. [Please click here to view a larger version of this figure.](#)

Figure 4: Posterior tympanotomy. (A) The facial nerve (FN) passes through the bony passage called the facial canal, and it runs between the posterior wall of the external auditory canal bone (PAC) and horizontal semicircular canal (HSC). The anterior region of the nerve is the facial recess (asterisk). (B) Make a hole by drilling the facial recess with a 0.6 mm diameter diamond bar. (C) By enlarging the hole, the round window niche (RWN) becomes visible. (D) Via the hole in the facial recess, it is possible to administer solution into the RWN (arrow) with a 25-gauge or thinner needle. [Please click here to view a larger version of this figure.](#)

Figure 5: Auditory brainstem response (ABR) assessment. Posterior tympanotomy was performed on a 7-year-old common marmoset, and 1 μ L of phosphate buffered saline was administered to the round window niche. ABRs were measured before and after surgery, and their representative waveforms are shown in (A) and (B) respectively. The waveforms and thresholds of the ABRs, evoked with tone burst in six frequencies, were comparable to a previous study^{17,18}. (C) There was no ABR threshold change from before to after surgery. [Please click here to view a larger version of this figure.](#)

Discussion

The volume of cochlear blood flow is extremely small, estimated to be in the order of 1/1,000,000 of the total cardiac output in humans, and access is limited by the presence of the blood-cochlea barrier which separates the inner ear from the general circulation^{19,20}. For these reasons, drug or viral vector administration into the inner ear is favorable, not via systemic administration but rather by direct administration. In mouse and guinea pig experiments, various approaches have been reported, such as the trans round window with post-auricular approach or ventral approach, cochleostomy, endolymphatic sac delivery, and semicircular canal approach^{21,22,23,24,25,26}.

In the present study, we used posterior tympanotomy and round window administration as a potential method for the translational research. Posterior tympanotomy is a well-established approach for a clinical surgical procedure and is suitable for common marmosets because the anatomical character of the tympanic cavity resembles that of humans, whereas its route mastoid is mostly composed of a large single cavity with very thin bone septa. Indeed, we performed all of the posterior tympanotomy procedure without any complications or hearing loss in the common marmoset. If posterior tympanotomy is performed, drug administration can be performed under the clear view around important tissues such as the semicircular canals, facial nerve, stapes, and round window niche. Of note, we can administer the solution via the basal turn of the cochlea, HSC, posterior semicircular canal, and round window niche with this single approach. Round window administration is, according to research using mice, less invasive and provides a more uniform solution expansion in cochlea perilymph than cochleostomy²⁷. It has also been reported that the direct injection of adenovirus via semicircular canal gene transfer showed transduction of genes predominantly in the vestibular organ²⁶. Endolymphatic sac delivery provides direct injection to endolymphatic space and is reported as the viral vector reached the vestibular end organ and cochlea²⁵. However the information about the efficiency seems inadequate.

To perform a safe operation, anatomical information is vital. The basic anatomical character of the middle ear tympanic cavity and the inner ear is similar when compared between humans and common marmosets. The important anatomical parts to perform the operation such as the junction between the middle ear cavity and the mastoid cavity, the position of ossicles, and the position of the facial nerve and chorda tympani are completely analogous to the human. However, there are some differences. The human mastoid cavity consists of many air cells and we need to drill these cells to approach the inner ear. This is called "mastoidectomy" in the clinical surgical procedure, and takes time and destroys the original morphology. On the other hand, the common marmoset mastoid cavity is almost a single cavity with very thin bone septa and the semicircular canals are larger and arch out into the mastoid cavity when compared to the human anatomy. These differences are advantageous for inner ear drug administration. When administering drugs to the endolymph directly, the semicircular canal approach is possible by making a small window to the HSC, as previously reported in other species²⁶. Although this approach was not performed in this experiment, the HSC of the common marmoset can be easily performed due to its size and easy detectability. However, before adopting the approach, the risk of lymphorrhea, which can induce hearing impairment, should be considered. We assume the semicircular canal approach is not an ideal

experimental system with which to assess hearing levels after an operation. In general, to prevent lymphorrhea, packing the window with adipose tissue and fibrin glue is effective.

In this case, we placed emphasis on a less invasive operation, and placed solution on the round window membrane without puncture. However, single administration does not seem effective to deliver a sufficient dose of a drug or viral vector to the perilymph. A simple solution for this problem is to make a hole in the round window membrane and inject the solution directly. As described above, however, making a hole in the inner ear causes lymphorrhea, which may possibly induce hearing loss and vestibular dysfunction in human patients, so this will be difficult to apply to a clinical setting. Also, unlike rodents, marmosets originally lived in trees, and move stereoscopically in a breeding cage, so vestibular dysfunction can also be a critical complication. Therefore, in the future, it will be important to improve the efficiency of drug diffusion without piercing the round window membrane. One possibility is using an osmotic pump and sustained preparation. An osmotic pump would provide continuous administration via a catheter whose tip is settled on the round window membrane. At present, ploxamer407 and gelatin hydrogels can be used as sustained preparations for round window membrane drug delivery. These devices and carriers are already being used in clinical trials^{28,29,30,31}.

In short, this surgical method allows us to perform the safe and accurate administration of drugs to the inner ear without hearing loss in a primate, which is of great importance in obtaining pre-clinical proof of concept for translational research.

Disclosures

The authors have nothing to disclose.

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