

Postaural injection is a systemic delivery supported by symmetric distribution of Gd-DOTA in both the ipsilateral and contralateral ears[☆]

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Abstract

Postaural injection of therapeutics was recently applied in clinical practice to treat inner ear diseases based on supposed existence of a direct channel from the postaural area to the inner ear. Doubting on the associated reports and aiming to provide evidence on the inner ear uptake mechanism, the present study tracked the dynamic distribution of gadolinium-tetra-azacyclo-dodecane-tetra-acetic acid (Gd-DOTA) in rat inner ears after postaural injection using MRI. A targeted tympanic medial wall delivery was utilized as control. The results showed that, at the early time points after postaural injection, Gd-DOTA distributed mainly in tissues surrounding the bulla, temporal bone and skull and neck space. In the inner ear, there was gradual uptake of Gd-DOTA on both the ipsilateral and contralateral sides with equal signal intensities. There was no sign of direct channel carrying the agent from the postaural area to the inner ear. Targeted tympanic medial wall delivery induced significantly greater uptake of Gd-DOTA in the inner ear than did postaural injection. At 30 min post-administration, targeted tympanic medial wall delivery yielded 4.6-folds higher signal intensity than did postaural injection. The total dose of Gd-DOTA delivered by the targeted tympanic medial wall approach was only 0.1% of that delivered by postaural injection. In conclusion, postaural injection is a systemic administration, which is similar to hypodermic injection, rather than a focal delivery method. By contraries, targeted tympanic medial wall delivery induces fast and abundant uptake of Gd-DOTA in the ipsilateral inner ear without significant distribution in unwanted areas. Copyright © 2016, PLA General Hospital Department of Otolaryngology Head and Neck Surgery. Production and hosting by Elsevier (Singapore) Pte Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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1. Introduction

Focal drug delivery in inner ear therapy, especially through transtympanic injection, has recently gained increasing popularity, partially because of its convenience, efficiency, and

reduced systemic drug exposure and associated adverse effects (Schuknecht, 1956; Zou et al., 2014). Alternatively, Yang et al. (2007) reported that postaural single injection of betamethasone was more effective in treating intractable low-frequency sensorineural hearing loss than oral administration of Merislon and Sibelium for two weeks (Yang et al., 2007). Subsequently, postaural injection of therapeutics was also employed to treat sudden deafness and tinnitus (Diao et al., 2013; Li et al., 2015; Wu et al., 2015). Potential existence of a direct transportation channel from the postaural area to the inner ear was reported to support the postaural injection approach (Lin and Yu, 2009). Postaural injection reportedly induced greater distribution of gadolinium chelate in the inner ear of guinea pigs than did intravenous injection (Li et al.,

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2012). The authors believed that postaural injection was a noninvasive method of focal drug delivery (Li et al., 2012).

I doubt on the reports associated with postaural injection. Based on anatomy, there is no direct transportation channel from the postaural area to the inner ear. Theoretically, postaural injection is a type of hypodermic injection that would never induce different distribution of agents in the inner ear between the ipsilateral and the contralateral sides. Defining postaural injection as a noninvasive method is conceptually incorrect because the needle has to penetrate the skin, which is an invasive procedure (Li et al., 2012). In order to support my opinion, the present study tracked the dynamic distribution of gadolinium-tetra-azacyclo-dodecane-tetra-acetic acid (Gd-DOTA) in the surrounding tissues and inner ear of rat after postaural injection by following a previously reported protocol (the reported concentration should be 0.5 mol/L instead of 0.5 mmol/L) (Li et al., 2012).

2. Materials and methods

Nine male Sprague Dawley rats, weighing between 266 g and 635 g, were provided by and maintained in the Biomedicum Helsinki Laboratory Animal Centre of the University of Helsinki. Four rats were assigned in the experimental group of postaural injection of Gd-DOTA and five rats were employed as controls receiving targeted tympanic medial wall delivery (Zou et al., 2011). All the animal experiments were approved by the Ethical Committee of the University of Tampere (permit number: LSLH-2006-4143/Ym23). Animal care and the experimental procedures were conducted in accordance with the pertinent European legislation. During the experiments, animals were anesthetized with isoflurane (Piramal Healthcare, Boise, USA) using a 5% isoflurane–oxygen mixture (flow-rate 1.0 L/min) for induction and 3% isoflurane–oxygen mixture for maintenance via a facemask with the animals' eyes protected with Viscotears® (Novartis Healthcare A/S, Denmark).

A 4.7 T MR scanner with a bore diameter of 155 mm (PharmaScan, Bruker BioSpin, Ettlingen, Germany) in combination with a dedicated rodent head coil (linear birdcage coil, diameter 38 mm) was used for *in vivo* MR measurements. The body temperature of the rat was maintained by circulating warm water and respiration was recorded using the Physio Tool-1.0.b.2 program (Bruker, Ettlingen, Germany). After postaural injection of Gd-DOTA on the left side at a dosage of 1.5 mmol/kg, the rat was placed in the MR scanner with ears positioned at the isocentre for imaging. In the control group, 2.5 μ l Gd-DOTA (0.5 mol/L) (0.0031 mmol/kg in average) was injected onto the tympanic medial wall of the left ear using the custom-made device that was previously reported and the rats were kept in a lateral position with the injected ear up for 15 min before imaging (Zou et al., 2011). After establishing inner ear geometry using a T2-weighted rapid acquisition with relaxation enhancement (RARE) sequence, the Gd-DOTA uptake in the inner ear was evaluated using a T1-weighted RARE sequence and a fluid-attenuated inversion-recovery (FLAIR) sequence for 2-dimensional (2D) and 3D

images as reported earlier (Zou et al., 2011, 2012a). The parameters for these sequences are as follows. RARE sequence for T2-weighted 2D images: TR/TE_{eff} 2500/40 ms, RARE factor 8, matrix size 256 \times 256, slice thickness 0.8 mm, FOV 5.0 \times 5.0 cm², resolution 0.195 \times 0.195 mm², NEX 3. RARE sequence for T1-weighted images: TR/TE_{eff} 500/10 ms, RARE factor 4, matrix size 256 \times 192, slice thickness 0.5 mm, FOV 2.5 \times 2.5 cm², resolution 0.098 \times 0.13 mm², NEX 33. RARE sequence for T1-weighted 3D images: TR/TE_{eff} 500/12 ms, RARE factor 16, matrix size 64 \times 64 \times 64, FOV 0.89 \times 0.89 \times 0.89 cm³, resolution 0.139 \times 0.139 \times 0.139 mm³, NEX 2. FLAIR sequence for 2D images: TR/TE_{eff} 8000/40 ms, inversion time 1800 ms, RARE factor 16, matrix size 256 \times 192, slice thickness 0.5 mm, FOV 3.0 \times 3.0 cm², resolution 0.117 \times 0.156 mm², NEX 7. In the group of postaural injection, distribution of Gd-DOTA from the injection site towards the surrounding area was followed from 5 min through 42 min after injection. Distribution of Gd-DOTA in the inner ear was followed from 45 min through 101 min after injection. MRI was repeated in one rat one week after the injection. Animals in the control group were imaged at the time points of 30 min, 43 min, 60 min, 70 min, 4 h, 6 h, and 2 d after administration.

The ParaVision PV 4.0 (Bruker, Ettlingen, Germany) software was used for post-processing and quantification of MR images. The IBM SPSS statistics 23 software was employed for statistical analysis. Signal intensities in the region of interests (the scala tympani, scala vestibuli and vestibulum), and the average values between the ipsilateral and contralateral ears during the period of 45–101 min were compared using paired-samples *t*-test. Signal intensities in the ipsilateral inner ear of the targeted tympanic medial wall delivery group at the time points of 30 min and 60 min were compared to that of the postaural injection group at time points of 45 min and 73 min using independent-samples *t*-test (this arrangement would not underestimate the inner ear signal in the postaural injection group because the uptake did not reach a plateau during the observation time of 101 min). *p*-values below 0.05 were considered statistically significant.

3. Results

Dynamic distribution of Gd-DOTA in the injection area and surrounding tissues is demonstrated in Fig. 1. Owing to T₂*-related signal loss (Hagberg and Scheffler, 2013), the MRI signal in the center of injection site (fluid), which mainly located hypodermically, was fully eliminated during the observation period of 42 min, but Gd-DOTA was always detected in the skin (Fig. 1A–D). At 5 min after postaural injection, Gd-DOTA distributed mainly in the tissues surrounding the bulla, temporal bone and skull on the ipsilateral side (Fig. 1A). At 42 min, Gd-DOTA arrived at the nearby space and the temporal bone and skull on the contralateral side (Fig. 1B and C). The signal in the inner ear was weak (Fig. 1D). In the control group of targeted tympanic medial wall delivery, Gd-DOTA was absent in the tissues surrounding the bulla. However, dynamic accumulation of Gd-DOTA in the

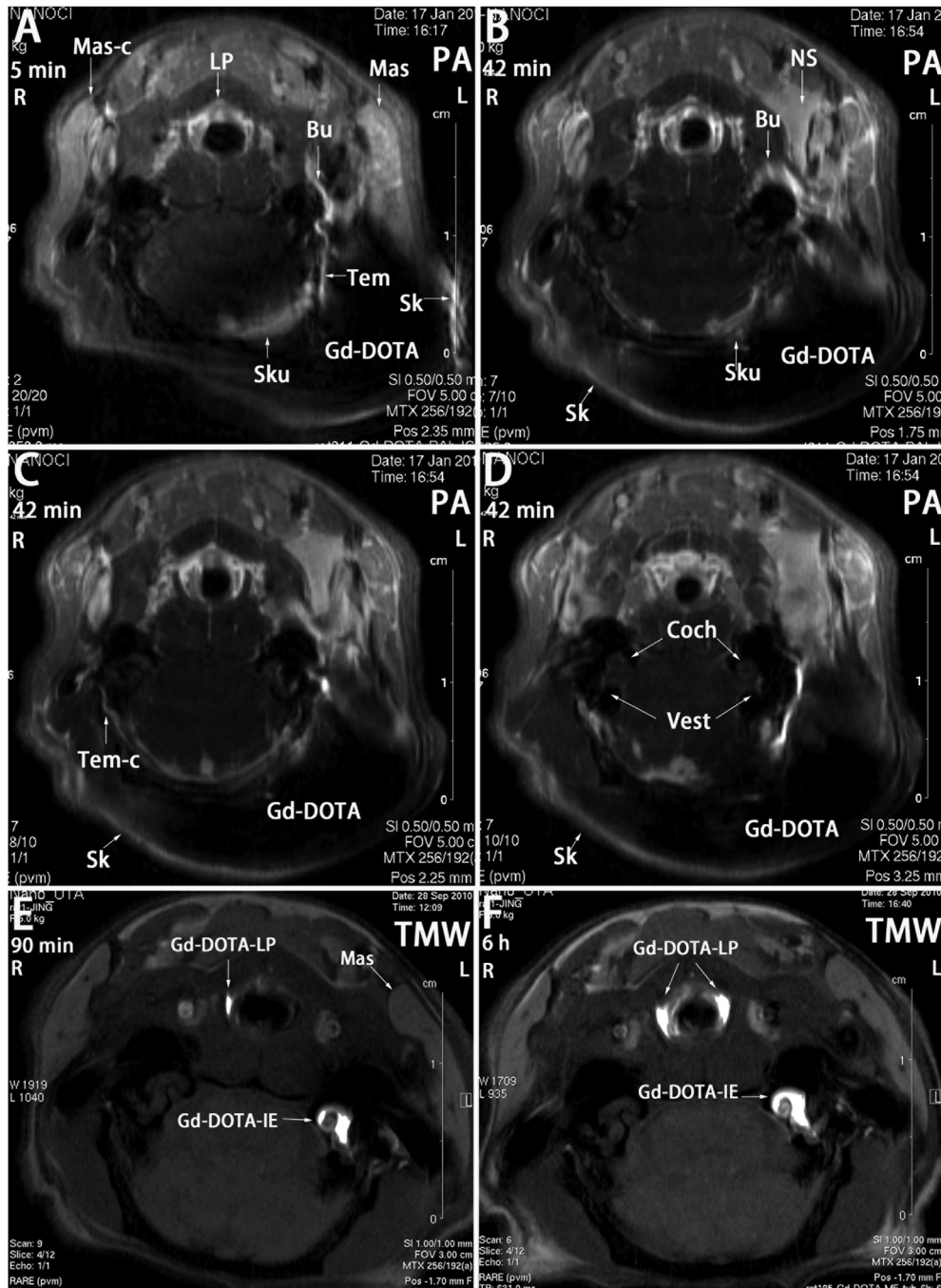


Fig. 1. Different distribution of Gd-DOTA in the surrounding area after postaural injection and targeted tympanic medial wall delivery in rats shown by MRI. Gd-DOTA was injected on the left side (L), and the right side (R) without injection was assigned as control. Two-dimensional T1-weighted RARE sequence was used to acquire the images at various time points. Bu: bulla; coch: cochlea; Gd-DOTA: Gd-DOTA liquid; Gd-DOTA-IE: Gd-DOTA in the inner ear; Gd-DOTA-LP: Gd-DOTA in the laryngopharynx; LP: laryngophaynx; Mas: masseter; Mas-c: masseter of the contralateral side; NS: neck space; Sk: skull; Tem: temporal bone; Tem-c: temporal bone of the contralateral side; Vest: vestibule. The scale bars on the left were created by the ParaVision PV 4.0 software.

laryngopharynx was observed during the period of 90 min through 6 h after administration (Fig. 1E and F).

In the inner ear, Gd-DOTA was equally taken up on both the ipsilateral and contralateral sides on images acquired using either the T2-weighted RARE or FLAIR sequence (Fig. 2). The MRI acquired using the FLAIR sequence demonstrated higher contrast effect than that using the T1-weighted RARE sequence. However, the latter demonstrated better anatomy

than did the former. There was an insignificant difference in the signal intensity between the ipsilateral and contralateral sides and the uptake of Gd-DOTA in the inner ear did not reach a plateau during the observation time of 101 min (Fig. 3). After one week, Gd-DOTA signal disappeared in the inner ear. In the control group of targeted tympanic medial wall delivery, intense signal was detected in the vestibule and lower turns of the cochlea even at the early time point of

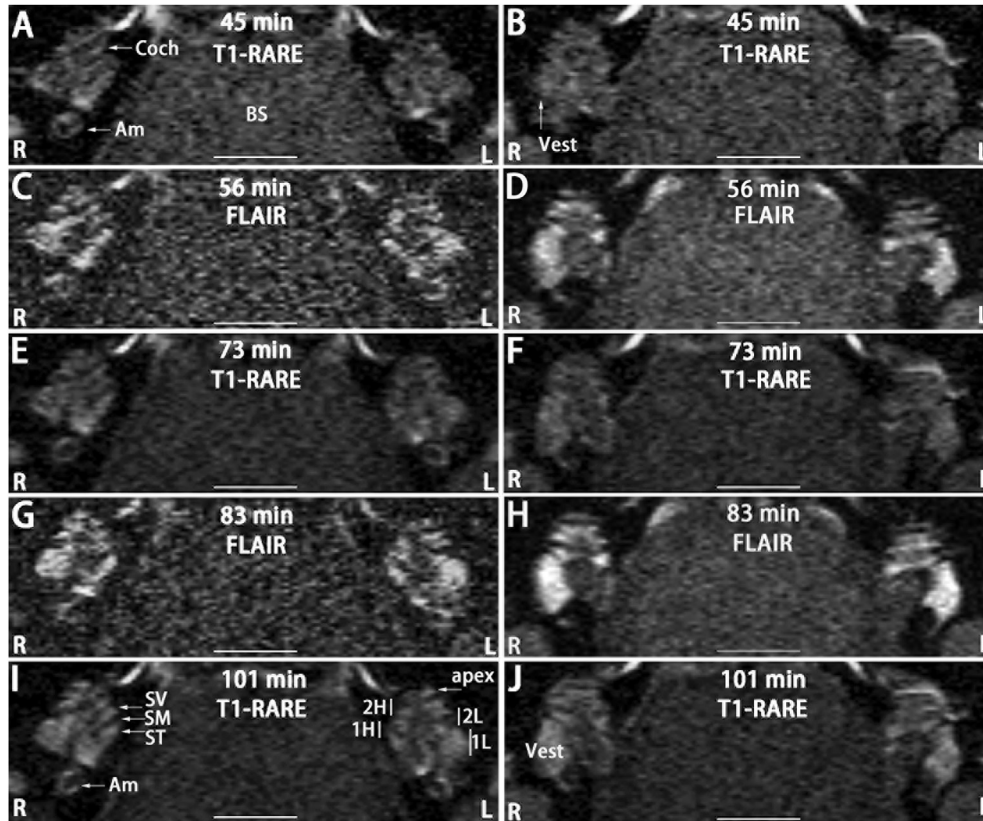


Fig. 2. Dynamic uptake of Gd-DOTA in rat inner ear after postaural injection detected by MRI. Gd-DOTA was injected on the left side (L), and the right side (R) without injection was assigned as control. Either 2-dimensional T1-weighted RARE or fluid-attenuated inversion-recovery (FLAIR) sequence was utilized to acquire the MRI. Am: ampulla; BS: brain stem; Coch: cochlea; L: left side; R: right side; SM: scala media; ST: scala tympani; SV: scala vestibuli, Vest: vestibulum; 1H: first higher turn; 1L: first lower turn; 2H: second higher turn; 2L: second lower turn. Scale bar = 5 mm.

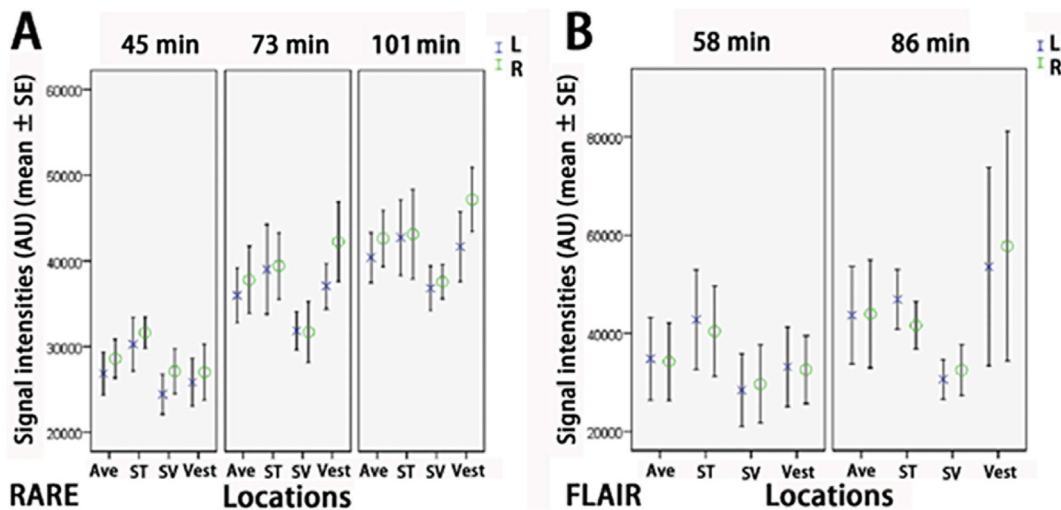


Fig. 3. Quantificational changes in the MRI signal intensities of the rat inner ear after postaural injection of Gd-DOTA. The images were acquired using either T1-weighted RARE (A) or FLAIR (B) sequence. There was insignificant difference between the injection side (left, L) and control side (right, R) ears at the defined time points ($p > 0.05$, paired-samples t-test). $n = 4$. Ave: average value of the signal intensities in the scala tympani (ST), scala vestibuli (SV) and vestibulum (Vest); AU: arbitrary unit.

30 min after administration (Fig. 4A–C). Gd-DOTA reached at apex of the cochlea at 60 min and evenly distributed in the cochlea at 70 min post-administration (Fig. 4F and G). Extremely strong signal was demonstrated in the cochlea at

4 h after delivery (Fig. 4H and I). Gd-DOTA appeared in the Eustachian tube on the ipsilateral side at 30 min and augmented at 60 min after delivery, indicating the secretion pathway of the excessive Gd-DOTA from the middle ear

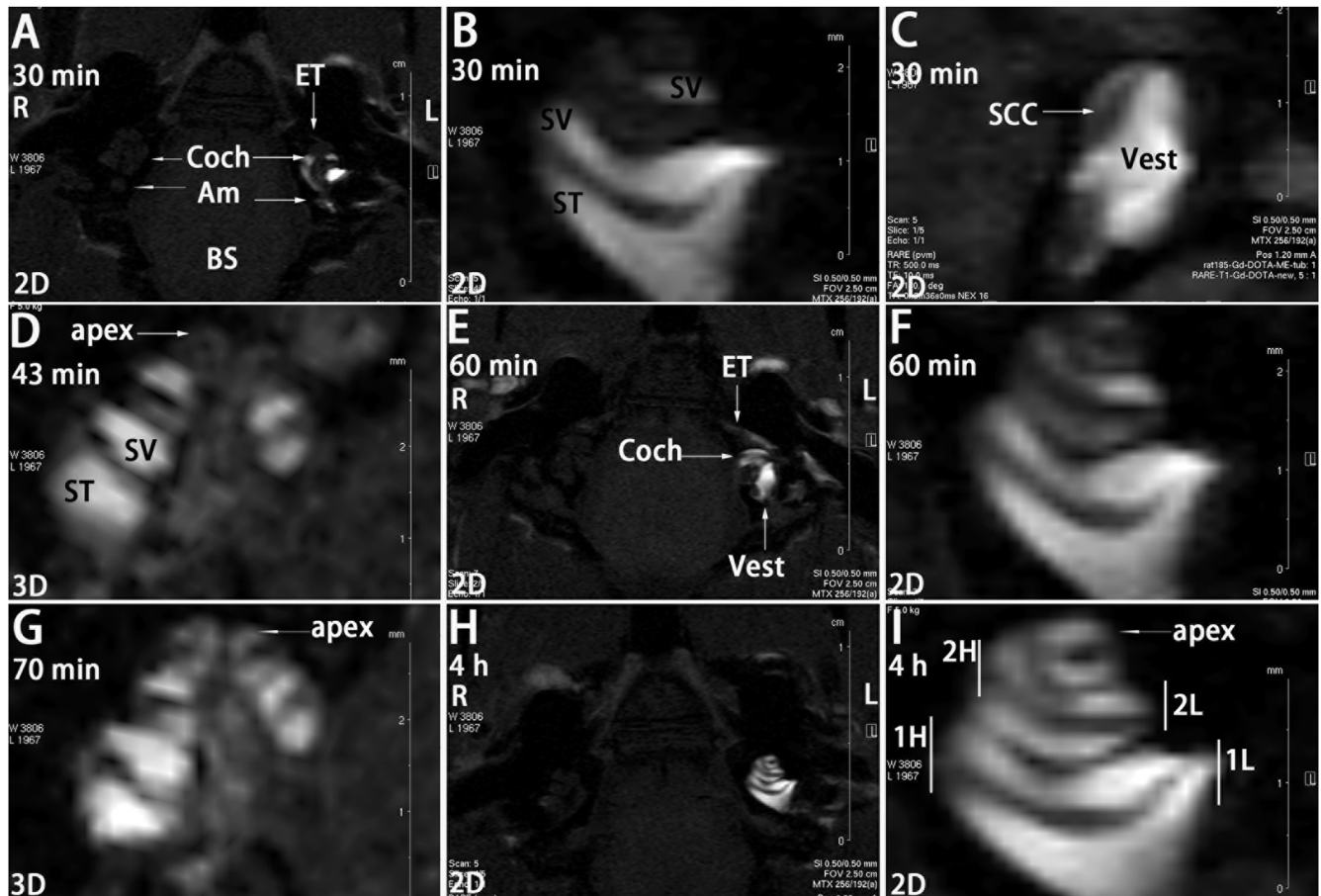


Fig. 4. Dynamic uptake of Gd-DOTA in rat inner ear after targeted tympanic medial wall delivery shown by MRI. Images were acquired using 2-dimensional (2D) and 3D T1-weighted RARE sequences. Am: ampulla; BS: brain stem; Coch: cochlea; ET: Eustachian tube; L: left side; R: right side; SCC: semicircular canal; ST: scala tympani; SV: scala vestibuli; Vest: vestibulum; 1H: first higher turn; 1L: first lower turn; 2H: second higher turn; 2L: second lower turn. The scale bars on the left were created by the ParaVision PV 4.0 software.

towards the laryngopharynx (Fig. 4A and E). The signal intensities in the ipsilateral inner ear of the targeted tympanic medial wall delivery group at the time points of 30 min and 60 min were significantly higher than those in the postauricular injection group at corresponding time points (Fig. 5). Regarding the average value in the vestibulum and scala vestibuli and tympani, signal intensity in the targeted tympanic medial wall delivery group at the time points of 30 min and 60 min was respectively 4.6-fold and 3.7-fold greater than those in the postauricular injection group. Notably, the total dose of Gd-DOTA delivered to individuals using the targeted tympanic medial wall approach was only 0.1% of that using postauricular injection. Gd-DOTA was almost undetectable from the ipsilateral inner ear 2 d after administration. There was no distribution of Gd-DOTA in the inner ear of the contralateral side during the experimental period of 2 d.

4. Discussion

Distribution of Gd-DOTA in the tissues surrounding the bulla at the earliest time after postauricular injection confirmed that the injection was accurate. The results that postauricular injection induced symmetric distribution of Gd-DOTA on both

the ipsilateral and contralateral sides in the inner ear suggest that postauricular injection is not a focal delivery method but a systemic administration similar to hypodermic injection (1867; Moge, 1953). It is strange that Li et al. defended that postauricular hypodermic injection is not a systemic delivery (Li et al., 2013). That the injected Gd-DOTA did not directly enter the inner ear from the postauricular area proved that there is not any direct channel from the postauricular area to the inner ear. Instead, Gd-DOTA entered the circulation system after diffusing in the surrounding tissues and spaces where there are plenty of capillary beds (lymph capillaries) to enable absorption of the delivered agents (Cornford and Oldendorf, 1993; DePace, 1981; Rose and Norris, 1990). Although there is no ethical problem to deliver therapeutics through postauricular injection from the scientific point of view, we should not expect any dramatic therapeutic effect super to other systemic administrations because the agents need to pass through the blood-perilymph barrier and reach the target cells in contact with the perilymph (Ulfendahl et al., 2000; Zou et al., 2003). The blood-perilymph barrier provides limited passages for almost all agents in order to maintain the homeostasis of the inner ear (Brac et al., 1984; Juhn et al., 1982; Lysaght et al., 2011; Swan et al., 2009). Injecting high dose of therapeutics

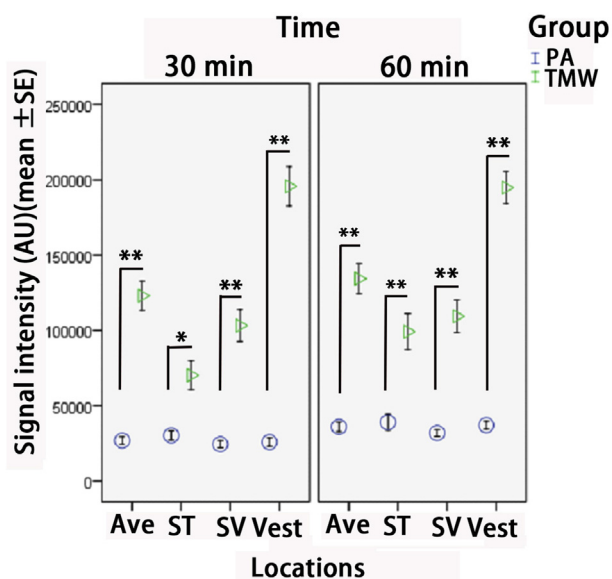


Fig. 5. Comparing the signal intensities in various inner ear area of the ipsilateral side after postaural injection and targeted tympanic medial wall delivery of Gd-DOTA in rats. MRIs were obtained using T1-weighted RARE sequence. 30 min: 30 min after targeted tympanic medial wall delivery (TMW) and 45 min after postaural injection (PA). 60 min: 60 min after TMW and 73 min after PA. * $P < 0.05$, ** $p < 0.01$ (independent-samples t-test). n = 4 (in PA) or 5 (in TMW). Ave: average value of the signal intensities in the scala tympani (ST), scala vestibuli (SV) and vestibulum (Vest); AU: arbitrary unit.

may induce sufficient loading of the agents in the inner ear, however this may cause associated adverse effects in other organs such as the kidney and liver.

Alternatively, focal intratympanic administration bypasses the blood-perilymph barrier and transports the agent into the inner ear through the round and oval windows (Duan et al., 2004; Schuknecht, 1956; Zou et al., 2014; Zou et al., 2011, 2012a, 2005, 2012b). Since agents are not diluted by the serum that occurs in systemic administration, a high concentration gradient of agents from the middle ear to the perilymph contributes to enrichment of agents in the inner ear transported across the round and oval windows. Intratympanic corticosteroids injections are reportedly beneficial in treating idiopathic sudden sensorineural hearing loss, Meniere's disease and noise-induced hearing loss (Filipo et al., 2013; Garduno-Anaya et al., 2005; Hong et al., 2009; Lavigne et al., 2015; Rauch et al., 2011; Zhou et al., 2013). In order to further reduce accumulation of agents in unwanted areas in individuals, we reported a targeted tympanic medial wall delivery method (Zou et al., 2011). Both Gd-DOTA and superparamagnetic iron nanoparticles have been administered in rats making use of this method (Zou et al., 2011, 2015a). We also observed efficient uptake of dimeglumine gadopentetate in the inner ear of patients with Meniere's disease after administration using this method (Zou et al., 2015b). The present study showed that there was insignificant distribution of Gd-DOTA in surrounding tissues and spaces while abundant uptake in the inner ear was achieved after targeted tympanic medial wall delivery. The average signal intensity in the inner

ear of the targeted tympanic medial wall delivery group at the time points of 30 min and 60 min was respectively 4.6-fold and 3.7-fold greater than those in the postaural injection group. However, the total amount of administered agents using the targeted tympanic medial wall approach was only 0.2% of that using postaural injection. It suggests that the targeted focal delivery method is more effective than postaural injection with the possibility of maximally avoiding adverse effects in the kidney and liver associated with drug delivery.

There are several additional scientific errors in the recently published article (Li et al., 2013). The statement that “there is no interaction between the *in vivo* contrast agent gadopentetate dimeglumine and the inner ear tissues” contradicts to the author's own results showing appearance of the contrast agent in the inner ear perilymph. The authors complained that intravenous injection did not induce endolymphatic distribution of agents. Actually, it is unnecessary for the therapeutics to enter the endolymph because the target cells are available to the perilymph. More importantly, drug delivery should not disrupt the endolymphatic space in order to maintain the inner ear function (Hibino and Kurachi, 2006; Rask-Andersen et al., 2006; Ulfendahl et al., 2000; Zou et al., 2013). The old term of “blood-labyrinth barrier” in the article should be updated to “blood-perilymph barrier and blood-endolymph barrier” because of their significantly different composition and biological functions (Zou et al., 2010b, 2010c). The updated terms of inner ear biological barriers are helpful for the clinician to understand the pharmaceutical mechanism of the inner ear therapy.

It is obvious that the physicochemical characteristics of Gd-DOTA is not identical to that of steroids. However, the uptake behavior of Gd-DOTA in the inner ear resembled that of steroids following either intravenous or intratympanic injection (Bird et al., 2011; Counter et al., 2000; Zou et al., 2010a, 2011, 2005, 2003, 2010b). This suggests that the results obtained using the MRI contrast agent of Gd-DOTA can be applied to predict the dynamics of steroids in the inner ear after similar administrations.

To conclude, postaural injection induced symmetric distribution of Gd-DOTA on both the ipsilateral and contralateral sides in the inner ear in addition to the surrounding tissues and spaces, proving that postaural injection is a systemic administration similar to hypodermic injection rather than a focal delivery method. By contrast, targeted tympanic medial wall delivery induced fast and abundant uptake of Gd-DOTA in the inner ear without significant distribution in unwanted areas.

References

- 1867. Report of the scientific committee appointed to investigate the physiological and therapeutic effects of the hypodermic method of injection. *Med. Chir. Trans.* 50, 561–643.
- Bird, P.A., Murray, D.P., Zhang, M., Begg, E.J., 2011. Intratympanic versus intravenous delivery of dexamethasone and dexamethasone sodium phosphate to cochlear perilymph. *Otol. Neurotol.* 32, 933–936.
- Brac, M.H., Lycett, P., McClure, J.A., 1984. Effect of high perilymphatic potassium on brainstem evoked response audiometric thresholds. *Acta Otolaryngol.* 97, 267–272.

- Cornford, M.E., Oldendorf, W.H., 1993. Terminal endothelial cells of lymph capillaries as active transport structures involved in the formation of lymph in rat skin. *Lymphology* 26, 67–78.
- Counter, S.A., Bjelke, B., Borg, E., Klason, T., Chen, Z., Duan, M.L., 2000. Magnetic resonance imaging of the membranous labyrinth during in vivo gadolinium (Gd-DTPA-BMA) uptake in the normal and lesioned cochlea. *Neuroreport* 11, 3979–3983.
- DePace, D.M., 1981. Morphologic study of the blood vessels of the superior cervical ganglion of the albino rat. *Acta Anat. (Basel)* 109, 238–246.
- Diao, M.F., Sun, J.J., Tian, F.J., Xu, S., Jia, Z.H., Liu, Y., Chen, D.L., 2013. [Effect of postauricular subcutaneous injection of triamcinolone acetone for subjective tinnitus]. *Zhonghua Yi Xue Za Zhi* 93, 3384–3387.
- Duan, M., Bjelke, B., Fridberger, A., Counter, S.A., Klason, T., Skjonsberg, A., Herrlin, P., Borg, E., Laurell, G., 2004. Imaging of the guinea pig cochlea following round window gadolinium application. *Neuroreport* 15, 1927–1930.
- Filipo, R., Attanasio, G., Russo, F.Y., Viccaro, M., Mancini, P., Covelli, E., 2013. Intratympanic steroid therapy in moderate sudden hearing loss: a randomized, triple-blind, placebo-controlled trial. *Laryngoscope* 123, 774–778.
- Garduno-Anaya, M.A., Couthino De Toledo, H., Hinojosa-Gonzalez, R., Pianese, C., Rios-Castaneda, L.C., 2005. Dexamethasone inner ear perfusion by intratympanic injection in unilateral Meniere's disease: a two-year prospective, placebo-controlled, double-blind, randomized trial. *Otolaryngol. Head Neck Surg.* 133, 285–294.
- Hagberg, G.E., Scheffler, K., 2013. Effect of r1 and r2 relaxivity of gadolinium-based contrast agents on the T1-weighted MR signal at increasing magnetic field strengths. *Contrast Media Mol. Imag.* 8, 456–465.
- Hibino, H., Kurachi, Y., 2006. Molecular and physiological bases of the K⁺ circulation in the mammalian inner ear. *Physiol. (Bethesda)* 21, 336–345.
- Hong, S.M., Park, C.H., Lee, J.H., 2009. Hearing outcomes of daily intratympanic dexamethasone alone as a primary treatment modality for ISSHL. *Otolaryngol. Head Neck Surg.* 141, 579–583.
- Juhn, S.K., Rybak, L.P., Fowlks, W.L., 1982. Transport characteristics of the blood–perilymph barrier. *Am. J. Otolaryngol.* 3, 392–396.
- Lavigne, P., Lavigne, F., Saliba, I., 2015 Jun 23. Intratympanic corticosteroids injections: a systematic review of literature. *Eur. Arch. Otorhinolaryngol.* [Epub ahead of print].
- Li, J.J., Yu, L.S., Xia, Y., Hao, F.B., Luo, W.L., Jing, Y.Y., 2012. Feasibility of postauricular administration facilitating drug entering the inner ear observed with 7.0 T MRI. *Chin. J. Otol.* 10, 144–148.
- Li, J., Yu, L., Xia, R., Gao, F., Luo, W., Jing, Y., 2013. Postauricular hypodermic injection to treat inner ear disorders: experimental feasibility study using magnetic resonance imaging and pharmacokinetic comparison. *J. Laryngol. Otol.* 127, 239–245.
- Li, X., Zhang, X.Y., Wang, Q.J., Wang, D.Y., 2015. Efficacy of methylprednisolone sodium succinate for injection (postotic injection) on the auditory threshold and speech recognition rate of sudden deafness patients. *Int. J. Clin. Exp. Med.* 8, 14110–14114.
- Lin, Y.J., Yu, L.S., 2009. Inner ear kinetics of dexamethasone after postauricular and intramuscular injections. *J. Otolaryngol. Head Neck Surg. China* 16, 381–384.
- Lysaght, A.C., Kao, S.Y., Paulo, J.A., Merchant, S.N., Steen, H., Stankovic, K.M., 2011. Proteome of human perilymph. *J. Proteome Res.* 10, 3845–3851.
- Mogey, G.A., 1953. Centenary of hypodermic injection. *Br. Med. J.* 2, 1180–1185.
- Rask-Andersen, H., Schrott-Fischer, A., Pfaller, K., Glueckert, R., 2006. Perilymph/modiolar communication routes in the human cochlea. *Ear Hear* 27, 457–465.
- Rauch, S.D., Halpin, C.F., Antonelli, P.J., Babu, S., Carey, J.P., Gantz, B.J., Goebel, J.A., Hammerschlag, P.E., Harris, J.P., Isaacson, B., Lee, D., Linstrom, C.J., Parnes, L.S., Shi, H., Slattery, W.H., Telian, S.A., Vrabec, J.T., Reda, D.J., 2011. Oral vs intratympanic corticosteroid therapy for idiopathic sudden sensorineural hearing loss: a randomized trial. *JAMA* 305, 2071–2079.
- Rose, E.H., Norris, M.S., 1990. The versatile temporoparietal fascial flap: adaptability to a variety of composite defects. *Plast. Reconstr. Surg.* 85, 224–232.
- Schuknecht, H.F., 1956. Ablation therapy for the relief of Meniere's disease. *Laryngoscope* 66, 859–870.
- Swan, E.E., Peppi, M., Chen, Z., Green, K.M., Evans, J.E., McKenna, M.J., Mescher, M.J., Kujawa, S.G., Sewell, W.F., 2009. Proteomics analysis of perilymph and cerebrospinal fluid in mouse. *Laryngoscope* 119, 953–958.
- Ulfendahl, M., Scarfone, E., Flock, A., Le Calvez, S., Conradi, P., 2000. Perilymphatic fluid compartments and intercellular spaces of the inner ear and the organ of Corti. *Neuroimage* 12, 307–313.
- Wu, S., Li, Q., Huang, S., 2015. [Clinical effect on the treatment of the low-middle frequency sudden hearing loss with postauricular injection of methylprednisolone]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 29, 928–930.
- Yang, X.Q., Yu, L.S., Ma, X., 2007. Postauricular injection of compound beta-methasone to treat the intractable low-frequency sensorineural hearing loss. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 42, 814–816.
- Zhou, Y., Zheng, G., Zheng, H., Zhou, R., Zhu, X., Zhang, Q., 2013. Primary observation of early transtympanic steroid injection in patients with delayed treatment of noise-induced hearing loss. *Audiol. Neurootol.* 18, 89–94.
- Zou, J., Pyykko, I., Counter, S.A., Klason, T., Bretlau, P., Bjelke, B., 2003. In vivo observation of dynamic perilymph formation using 4.7 T MRI with gadolinium as a tracer. *Acta Otolaryngol.* 123, 910–915.
- Zou, J., Pyykko, I., Bjelke, B., Dastidar, P., Toppila, E., 2005. Communication between the perilymphatic scalae and spiral ligament visualized by in vivo MRI. *Audiol. Neurootol.* 10, 145–152.
- Zou, J., Ramadan, U.A., Pyykko, I., 2010a. Gadolinium uptake in the rat inner ear perilymph evaluated with 4.7 T MRI: a comparison between transtympanic injection and gelatin sponge-based diffusion through the round window membrane. *Otol. Neurotol.* 31, 637–641.
- Zou, J., Zhang, W., Poe, D., Zhang, Y., Ramadan, U.A., Pyykko, I., 2010b. Differential passage of gadolinium through the mouse inner ear barriers evaluated with 4.7T MRI. *Hear Res.* 259, 36–43.
- Zou, J., Zhang, W., Poe, D., Qin, J., Fornara, A., Zhang, Y., Ramadan, U.A., Muhammed, M., Pyykko, I., 2010c. MRI manifestation of novel superparamagnetic iron oxide nanoparticles in the rat inner ear. *Nanomed. (Lond.)* 5, 739–754.
- Zou, J., Yoshida, T., Ramadan, U.A., Pyykko, I., 2011. Dynamic enhancement of the rat inner ear after ultra-small-volume administration of Gd-DOTA to the medial wall of the middle ear cavity. *ORL J. Otorhinolaryngol. Relat. Spec.* 73, 275–281.
- Zou, J., Poe, D., Ramadan, U.A., Pyykko, I., 2012a. Oval window transport of Gd-DOTA from rat middle ear to vestibulum and scala vestibuli visualized by in vivo magnetic resonance imaging. *Ann. Otol. Rhinol. Laryngol.* 121, 119–128.
- Zou, J., Sood, R., Ranjan, S., Poe, D., Ramadan, U.A., Pyykko, I., Kinnunen, P.K., 2012b. Size-dependent passage of liposome nanocarriers with preserved posttransport integrity across the middle-inner ear barriers in rats. *Otol. Neurotol.* 33, 666–673.
- Zou, J., Zhang, Y., Zhang, W., Poe, D., Zhai, S., Yang, S., Pyykko, I., 2013. Mitochondria toxin-induced acute cochlear cell death indicates cellular activity-correlated energy consumption. *Eur. Arch. Otorhinolaryngol.* 270, 2403–2415.
- Zou, J., 2014. Focal drug delivery in inner ear therapy. In: Domb, A.J., Khan, W. (Eds.), *Focal Controlled Drug Delivery*. Springer, London, UK., pp. 215–224.
- Zou, J., Ostrovsky, S., Israel, L., Lellouche, J.-p.M., Pyykko, I., 2015a. Targeted delivery of contrast agents to the tympanic medial wall at minimum amount and the efficient uptake in the inner ear through oval and round windows. *Austin J. Radiol.* 2, 1034.
- Zou, J., Peng, R., Zheng, G., Zhang, Q., Toppila, E., Zheng, H., Pyykko, I., 2015b. Improvement in the inner ear symptoms of patients with Meniere's disease after treatment using low-frequency vibration: a preliminary report. *Eur. Arch. Otolaryngol.* 2, 5–13.