rhIGF-1を蝸牛に持続投与したところ，有毛細胞保護効果と聴力改善がみられた(ラット[10]，モルモット[9])．これらを背景に，我々は急性感音難聴のステロイド無効例に対するIGF-1徐放ハイドロゲルによるフェーズⅠ/Ⅱ臨床試験を行なっている．

対象症例は，突発性難聴を含む急性感音難聴の標準治療であるステロイド全身投与を行なって無効であった成人で，発症後30日以内，他治療を受けていないなどの条件を満たすものである．鼓膜切開を行ない(**図5A**)，細径中耳内視鏡で正円窓窩・正円窓膜を確認し(**図5B**)，ここにrhIGF-1を含浸させたゼラチンハイドロゲルを留置する．目標症例数25例に対し，20例に投与を完了し(平成21年4月末現在)，プロトコル治療と関連する重篤な有害事象はなく，一部の症例では聴力の改善を認めている(論文投稿中)．

本臨床試験は，ヒトにおいて内耳に成長因子を投与する初めての試みである．rhIGF-1の内耳治療における効果を評価するだけではなく，今後の同様の内耳DDSの臨床試験を行なう場合のテンプレートとなり得るという点でも意義が大きい．

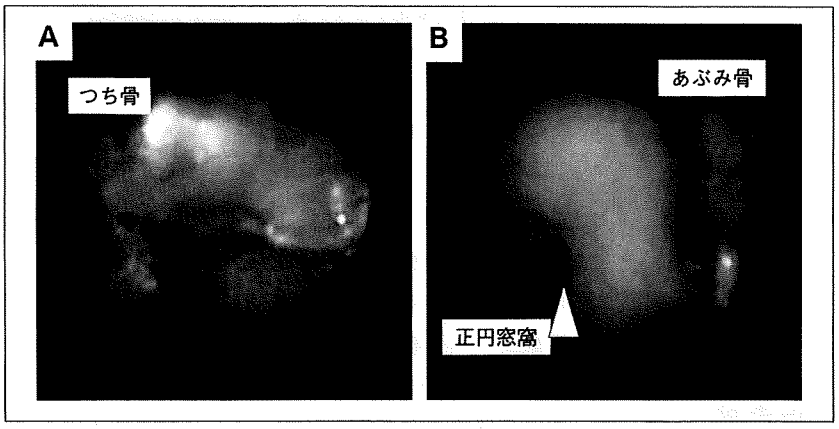
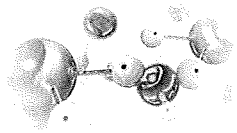


図5．正円窓へのアプローチ
細径内視鏡を用いて，鼓膜切開開窓部(A)から鼓室内を観察すると，あぶみ骨や正円窓窩が確認できる(B)．

リドカイン徐放パーティクルによる耳鳴治療

耳鳴は，体外の明らかな音源なしに知覚される音と定義され，非常にありふれていて，我慢ならない自覚症状である．難聴とともに出現することが多いとされているためこの治療をまず考慮するが，多くの場合に問題となる感音難聴で前述のように急性期にしか積極的な治療手段が無く，それも無効な症例もあり，結局耳鳴が遷延することになる症例も多い．慢性的な耳鳴の場合，不眠や抑うつ状態を引き起こし，病院ショッピングや悪徳業者による詐欺など様々な社会問題とも関連することになり得るが，耳鳴を抑制する現実的な治療法はない．そのなかで，局所麻酔薬リドカインに耳鳴抑制効果があることは経験的に知られていた．全身投与での有効性は二重盲検でも示されているもの[26)27)]，持続時間が数時間以内と非常に短く，めまいや不整脈などの副作用があり得るために一般的な治療とはなっていない．また局所投与(鼓室内投与)でも高い有効性が示されているが[28)29)]，内耳麻酔による非常に強いめまいが発生することから，治療として受け入れがたいものであった．我々は，内耳DDSを用いることで経正円窓膜的に濃度を制御して持続的に投与できると考え，リドカイン含有PLGAマイクロパーティクルを作成して*in vitro*での徐放性を確認，モルモットの正円窓膜上にパーティクルを留置してモルモットの蝸牛内でリドカインが数日にわたって検出できること，眼振が起きないことなどを確認した(larryngoscope)(in print)．耳鳴は自覚症状であるため動物実験は容易ではないが，行動モデルを用いた実験系が報告されており[30)31)]，これらを用いた効果



の確認をするとともに，慢性耳鳴症例を対象とした臨床試験を準備中である．

内耳DDSの将来

内耳DDSは，研究面でも臨床面でもようやく動き始めたところである．例えば，正円窓膜を経由した内耳DDSにおいて決定的なファクターである正円窓膜の透過性についての評価はまだ十分ではなく，これを向上させることで投与効率の向上が期待できる．従来型のPLGAナノパーティクルでもパーティクル自体が正円窓膜を透過することがわかっているが[12]，NANOEAR (http://www. nanoear. org/)というヨーロッパ諸国を中心としたプロジェクトでは，様々に修飾したナノパーティクルの使用を試みている．また，全身投与による内耳ターゲティングにはこれからの技術的なブレイクスルーが望まれるが，我々はステルス型ナノパーティクルによるステロイド投与について検討中である(投稿準備中)．

内耳DDSを用いて何をするかという点は，今後更に重要になる．いったん失われた聴力を回復させることが難しい根本的な理由の1つは，いったん失われた内耳有毛細胞が再生しないことである．発生学研究の中でも内耳の発生は現在ホットな領域であり，これを応用した内耳再生治療が現実のものとして近づいている．例えば，Notchシグナルの阻害薬であるγセクレターゼ阻害薬を内耳に投与すると新たな有毛細胞が発生することや[32)-34)]，bHLH型転写因子Atoh1を強制発現することで傷害された有毛細胞が再生することなどが報告されている[35]．例えば，これらを内耳DDSで安全に蝸牛に導入することができるなら，有毛細胞の再生の可能性がある．

別の観点では，内耳の画像化技術への応用が考えられる．難聴治療の問題点として，難聴の非侵襲的な部位診断が困難であることを挙げた．現在，マイクロCT[36)37)]やマイクロMRI[38]を用いた蝸牛内構造の可視化が試みられているが，臨床的に用いる段階には至っていない．内耳DDSを用いて内耳形態の描出，あるいはカリウムイオンやpHの可視化ができれば，難聴の病態診断となり得る．

内耳DDSは，聴覚障害に対する治療開発の臨床応用というアウトプットへの鍵となる因子である．これから更なる広がりが期待される．

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The Laryngoscope

Sustained Delivery of Lidocaine Into the Cochlea Using Poly Lactic/Glycolic Acid Microparticles

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Objectives/Hypothesis: Lidocaine is a local anesthetic that is known to suppress tinnitus via systemic or local application; however, this effect has only limited duration. The current study aimed to establish a method for the sustained delivery of lidocaine into the cochlea using poly lactic/glycolic acid (PLGA) microparticles.

Study Design: Experimental study.

Methods: Lidocaine-loaded PLGA microparticles were produced and their in vitro-release profiles were examined. The lidocaine concentrations in the perilymph were measured at different time points following the application of the lidocaine-loaded PLGA microparticles to the round-window membranes of guinea pigs. The possible adverse effects of the local application of lidocaine-loaded PLGA microparticles were also examined.

Results: The in vitro analyses revealed that the microparticles were capable of the sustained delivery of lidocaine. The in vivo experiments demonstrated the sustained delivery of lidocaine into the cochlear fluid, and the maintenance of high lidocaine concentrations in the perilymph for up to 3 days after application. Nystagmus and inflammation in the middle ear mucosa were not detected after the local application of lidocaine-loaded PLGA microparticles, although temporary hearing loss was observed.

Conclusions: Lidocaine-loaded PLGA microparticles were shown to be capable of the sustained delivery of lidocaine into the cochlea, suggesting that they could be used for the attenuation of peripheral tinnitus.

Key Words: Biomaterial, drug-delivery system, inner ear, poly lactic/glycolic acid, tinnitus.

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INTRODUCTION

Tinnitus is the perception of sound within the human ear in the absence of corresponding external stimuli. Tinnitus is not a disease, but rather a symptom resulting from a range of underlying causes. It can be a serious problem for patients, because it can lead to severe depression with negative effects on the activities of daily life. Tinnitus is encountered by otolaryngologists worldwide; however, at present, only limited treatment options are available. Many different drugs have been used for the treatment of tinnitus, but with little success. One of the major obstacles to developing efficient treatments for tinnitus is the fact that tinnitus has various forms and underlying mechanisms. Theories about tinnitus pathophysiology emphasize abnormal peripheral or central neural activity in the auditory system.[1]

Several studies have reported on the efficacy of the local or systemic administration of lidocaine,[2–4] which is the most commonly used local anesthetic and is also employed as an antiarrhythmic agent. Various reports have suggested the cochlea, auditory nerve, and central auditory pathway as sites of lidocaine action on tinnitus.[5] Several studies have shown that the systemic application of lidocaine can alleviate tinnitus; however, this effect has only limited duration, and the treatment carries a risk of serious side effects, including cardiac arrhythmia and central nervous system excitation or

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depression.[2] The local application of lidocaine into the intratympanic space can avoid such systemic toxicity, but causes vertigo or dizziness.[4] Tinnitus suppression by local lidocaine application also has limited duration.[3]

Rapid recent technological progression has made possible the sustained and/or targeted delivery of drugs. We have developed a local system for the sustained delivery of growth factors for the treatment of inner ear disorders.[6] Local application can eliminate systemic side effects and deliver drugs to targeted organs at high concentrations. The use of drug-delivery systems in local treatment prolongs the therapeutic effects, and is sometimes necessary to achieve biological effects. We proposed that the use of drug-delivery systems for local lidocaine application could help to prolong the suppression of tinnitus caused by cochlear dysfunction. The current study thus aimed to develop a system for the sustained delivery of lidocaine to the cochlea, and to examine the possible adverse effects. Poly lactic/glycolic acid (PLGA), which is a material used for absorbable sutures, was investigated as a biomaterial for the sustained delivery of lidocaine. PLGA microparticles encapsulating lidocaine were produced, and their release profiles were examined both in vitro and in vivo. The effects on hearing, vestibular function, and histology of the middle ear mucosa were determined to evaluate the risk of adverse effects.

MATERIALS AND METHODS

Animals

Hartley guinea pigs (female; weight, 300–500 g; N = 74) were purchased from Japan SLC Inc. (Shizuoka, Japan). The Animal Research Committee of the Graduate School of Medicine, Kyoto University, Kyoto, Japan, approved all experimental protocols. Animal care was supervised by the Institute of Laboratory Animals of the Graduate School of Medicine, Kyoto University. All experimental procedures were performed in accordance with the National Institutes of Health, Guidelines for the Care and Use of Laboratory Animals.

Preparation of Lidocaine-Loaded PLGA Microparticles

PLGA with a lactic/glycolic acid ratio of 70:30 (molecular weight, 10,000 Daltons) was obtained from Polysciences Inc. (Warrington, PA). Polyvinyl alcohol (PVA), UP180 (degree of polymerization, 1,800), and UMR10H (degree of polymerization, 250) were purchased from Japan Vam & Plval Co., Ltd. (Osaka, Japan). Lidocaine powder and phosphate buffered saline (PBS) were purchased from Nakarai Tesque (Kyoto, Japan). Acetonitrile and Tween-80 were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Dichloromethane (DCM) was purchased from Fisher Scientific (Tokyo, Japan). All solvents were high-performance liquid chromatography (HPLC) grade.

Microparticles were prepared using emulsification by the homogenization-solvent evaporation method. Briefly, an organic phase was prepared consisting of the polymer (PLGA) and the drug (lidocaine), dissolved in an organic solvent (DCM). The organic phase was added to an aqueous phase containing a surfactant (PVA) to form an emulsion. This was broken down into microdroplets by applying external energy, and the droplets formed microparticles on solvent evaporation.

The effect of lidocaine loading on the release profile was examined in two kinds of PLGA microparticles. The microparticles were produced using 2 g PLGA or 2 g lidocaine dissolved in 5.5 mL DCM, or 500 mg PLGA or 500 mg lidocaine dissolved in 5 mL DCM, respectively. The organic phase was then mixed with 50 mL of 1% (wt/wt) PVA solution, and 50 mL of 1% (wt/wt) PVA solution was added as the aqueous phase. This was followed by stirring at 5,000 rpm for 5 minutes, and evaporation of the solvent for 3 days. The microparticles were collected by centrifugation at 5,000 rpm and 4°C, and then freeze dried. The lidocaine-free microparticles were prepared using a similar method, but in the absence of lidocaine. The surface morphology and average particle diameters of the microparticles were examined by scanning electron microscopy (SEM). The average size was estimated from the diameter of 100 randomly selected microparticles. Finally, lidocaine-loaded large (Lido-L) microparticles and lidocaine-loaded small (Lido-S) microparticles were prepared.

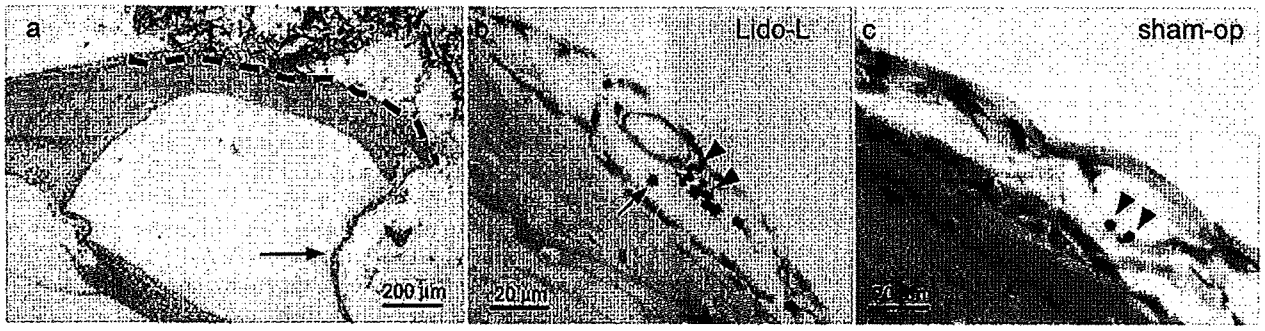
The lidocaine loading contents of the Lido-L and Lido-S microparticles were analyzed by the method reported previously.[7] The concentration of lidocaine in the supernatant was measured by an ultraviolet (UV) detector (wavelength, 263 nm), and the total amount of lidocaine in the particles was determined. The lidocaine contents (%) were calculated as follows: (weight of lidocaine in microparticles)/(total weight of microparticles)×100. Three independent measurements were performed for each condition.

In Vitro-Release Profile

The lidocaine-release profiles of the Lido-L and Lido-S microparticles were determined in vitro. A 2.5-mg sample of the microparticles was incubated in a tube containing 1.5 mL PBS (pH 7.4) with 0.2% (wt/wt) Tween-80 buffer. The samples were then placed in a shaken water bath regulated at 30 rpm and 37°C. Sampling of 1 mL of the buffer was performed at 1, 2, 4, 6, and 12 hours, and 4, 7, 14, 21, 28, and 32 days after incubation, followed by replacement with 1 mL of fresh buffer. The concentration of lidocaine in the samples was determined using a UV detector (wavelength, 263 nm). The cumulative amount of lidocaine released was calculated for each time point. The lidocaine-release profile of each particle (%) was calculated as follows: (cumulative amount of lidocaine released)/(total lidocaine content)×100. The amounts of released lidocaine per hour were also calculated for the following time points: 0 to 1 hour, 1 to 2 hours, 2 to 4 hours, 4 to 6 hours, 6 to 12 hours, 12 hours to 4 days, 4 to 7 days, 7 to 14 days, and 14 to 21 days. Three independent measurements were performed for each condition.

In Vivo-Release Profile

The lidocaine concentration in the perilymph was measured on days 1, 3, 7, and 14 after the application of 2.5 mg Lido-L microparticles to the round-window membrane (RWM) of the guinea pigs (n = 16). The animals were anesthetized with an intramuscular injection of midazolam (5 mg/kg; Astellas Co., Tokyo, Japan), medetomidine (18.5 μg/kg; Zenoac, Fukushima, Japan), and butorphanol tartrate (0.25 mg/kg; Bristol-Myers, K.K., Tokyo, Japan). A small hole was made in the bulla to expose the cochlea, and Lido-L microparticles were placed on the RWM. To measure the lidocaine concentrations in the perilymph, the cochleae were excised from the temporal bones under general anesthesia on day 1 (n = 7), day 3 (n = 4), day 7 (n = 6), and day 14 (n = 4). Each cochlea was punctured at the apical portion, and 3 μL perilymph was aspirated using a 30-gauge needle (BD and Company, Fukuoka, Japan). The perilymph samples collected from the animals on day 1 after the sham-operation (n = 4) were used as a negative control.



AQ5 Fig. 1. Histopathology of the middle ear mucosa 7 days after local application of Lido-L microparticles (Lido-L) or sham-operation (sham-op). (a) The dotted line indicates the evaluated area of the middle ear mucosa. The arrow shows the RWM. (b) The middle ear mucosa treated with Lido-L microparticles showed a few lymphocytes (arrowheads) and a neutrophil (an arrow). (c) A few lymphocytes (arrowheads) were found in a sham-operated specimen.

The concentration of lidocaine in the perilymph was measured by reverse-phase (RP)-HPLC using the Shiseido Nanospace SI-2 system (Shiseido, Tokyo, Japan). The lidocaine was separated on a Cosmosil C_{18}-AR-II reverse-phase column AQ2 (5 μm, 4.6 mm I.D. × 150 mm; Nacalai Tesque, Kyoto, Japan) at 35°C. The mobile phase consisted of water (0.1 M NaH_2PO_4/Na_2HPO_4, pH adjusted to 4.5) and methanol at a volume ratio of 40:60. The flow rate was 0.3 mL/minute. The detector potential was maintained at +1.0 V.

Effects on Auditory Function

To assess the effects of the local application of Lido-L microparticles on the auditory function, the auditory brainstem response (ABR) was recorded. Lido-L microparticles (2.5 mg) were placed on the left RWM of guinea pigs (n = 4) under general anesthesia. Lidocaine-free PLGA particles were applied to the controls (n = 4). ABR measurements were performed preoperatively (day 0), and on days 1, 3, 7, and 14 after application, according to previous studies.[6]

Effects on Vestibular Function

Vestibular dysfunction was assessed by the occurrence of nystagmus after the local application of either Lido-L microparticles (n = 16) or a piece of gelatin sponge immersed with 20 μL of 0.5%, 1%, 2%, or 4% lidocaine hydrochloride (Astra Zeneca K.K., Osaka, Japan; n = 2 for each concentration of lidocaine). The Lido-L microparticles (2.5 mg) or pieces of gelatin sponge immersed with lidocaine hydrochloride were placed on the RWM of the right ear under general anesthesia with sevoflurane (Abbott Japan Co., Ltd., Tokyo, Japan). The direction and the duration of nystagmus were recorded using an infrared video system in a dark room for 2 hours. In addition, for the Lido-L microparticle-treated animals, the occurrence of nystagmus was evaluated on days 1, 3, and 7 after application. The concentrations of lidocaine in the perilymph were also measured 5 minutes after the local application of 1% or 2% lidocaine hydrochloride (n = 4 for each concentration of lidocaine). The concentration of lidocaine was analyzed using the method described for the in vivo-release profile.

Inflammatory Responses

The inflammatory responses in the middle ear following the local application of Lido-L microparticles were estimated histologically. On day 7 after the local application of Lido-L microparticles (2.5 mg) to the RWM of guinea pigs, the temporal bones were collected and fixed with 4% paraformaldehyde in PBS at pH 7.4 for 3 hours at room temperature (n = 4). Specimens obtained from sham-operated animals (n = 4), which underwent surgical procedures without substances being applied to the RWM, served as controls. The paraffin-embedded tissues were processed into 3 μm sections, and stained with hematoxylin and eosin. Two midmodiolar sections from each cochlea were subjected to quantitative analyses. The numbers of lymphocytes, neutrophils, and plasmacytes in the middle ear mucosa were counted in five randomly selected fields located within 1 mm of the edge of the RWM under a ×40 objective lens for each section (Fig. 1a). The distance from the edge of the AQ3 RWM was measured using Image J software (http://www.nist.gov/lispix/imlab/prelim/dnld.html). Cell counting was performed in a blind manner. The average cell number in five fields was used for each animal in the analysis.

Statistical Analyses

The differences in lidocaine concentrations among time points after Lido-L microparticle application were examined by analysis of variance (ANOVA) with the Tukey-Kramer test. The overall effect of the local application of Lido-L or lidocaine-free PLGA microparticles on the ABR thresholds was examined by 2-way factorial ANOVA. When the interactions were significant, multiple comparisons using the Tukey-Kramer test were performed for pair-wise comparisons. The numbers of infiltrated cells in the middle ear mucosa of the Lido-L microparticle-treated animals were compared with those in the sham-operated animals using the unpaired t test. A P value <0.05 was considered statistically significant. All data are represented as the mean ± standard error.

RESULTS

In Vitro-Release Profile

SEM demonstrated that the Lido-L and Lido-S microparticles had a smooth and round surface morphology (Fig. 2). The average outer diameter of the Lido-L F2 microparticles was 100.0 ± 3.0 μm, and that of the Lido-S microparticles was 5.0 ± 0.5 μm, indicating their stable production. The loading contents of lidocaine were 41.8% AQ4 ± 1.1% for the Lido-L microparticles and 5.0% ± 0.1% for the Lido-S microparticles. In general, the drug-release profiles of PLGA particles are partitioned into four phases: an initial burst period (phase 1), an induction period (phase 2), a slow-release period (phase 3), and a final release period (phase 4). The cumulative release

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Fig. 2. Surface morphology of lidocaine-loaded microparticles. The scale bar represents 20 μm.

profiles of lidocaine from the Lido-L and Lido-S microparticles are shown in Figure 3a. A burst period (phase 1) was observed for both types of microparticle at 24 hours, which was followed by phase 2. The Lido-L microparticles released 77.3% ± 2.3% of the lidocaine contents during phases 1 and 2 (days 0–7). By the end of phase 3 (days 7–56), 82.7% ± 3.3% of the lidocaine had been released. A final release period was not observed during the experimental period. The Lido-S microparticles released 76.6% ± 1.0% of the lidocaine during phases 1 and 2 (days 0–7). By the end of phase 3 (days 7–21), 94.7% ± 0.9% of the lidocaine had been released, and by the end of phase 4 (days 21–56) the amounts reached up to 98.9% ± 0.9%. The amounts of lidocaine released per hour from the Lido-L and Lido-S microparticles are shown in Figure 3b. The Lido-L microparticles exhibited the stable release of lidocaine during the initial 12 hours. In the first hour, lidocaine was released from these microparticles at a rate of 669.8 μg/hour. Between 1 and 6 hours, over 200 μg/hour lidocaine was released, whereas the rate fell to 79.7 μg/hour between 6 and 12 hours. Lidocaine was released at a rate of 10.5 μg/hour between 12 hours and 4 days, and this decreased to 2.4 μg/hour between 4 and 7 days. By contrast, the Lido-S microparticles released lidocaine at a rate below 30 μg/hour, even between 1 and 2 hours. These findings indicated that the Lido-S microparticles released the majority of the loaded lidocaine in an initial burst, which was not advantageous for sustained release. By contrast, the Lido-L microparticles showed the continuous release of comparatively large amounts of lidocaine during the initial 12 hours, indicating that local application might maintain high concentrations of lidocaine in the cochlear fluid for a few days. We therefore used Lido-L microparticles in the subsequent experiments.

In Vivo-Release Profile

RP-HPLC analyses demonstrated measurable concentrations of lidocaine in the perilymph samples collected from the cochleae 1 to 14 days after Lido-L microparticle application to the RWM (Fig. 4). The lidocaine concentrations were 744.0 ± 176.9 ng/mL on day 1, and 863.3 ± 366.3 ng/mL on day 3. The values decreased to 87.2 ± 27.2 ng/mL on day 7, and were maintained at 30.3 ± 13.6 ng/mL on day 14. The difference in lidocaine concentrations between days 3 and 7 was statistically significant (Tukey-Kramer test). These findings demonstrated that the lidocaine released from Lido-L microparticles was transferred into the perilymph through the RWM, and that high concentrations of lidocaine in the perilymph were maintained for at least 3 days, which was consistent with the in vitro-release profiles of the Lido-L microparticles.

Effects on Auditory Function

The ABR thresholds after the application of Lido-L or lidocaine-free microparticles are shown in Figure 5.

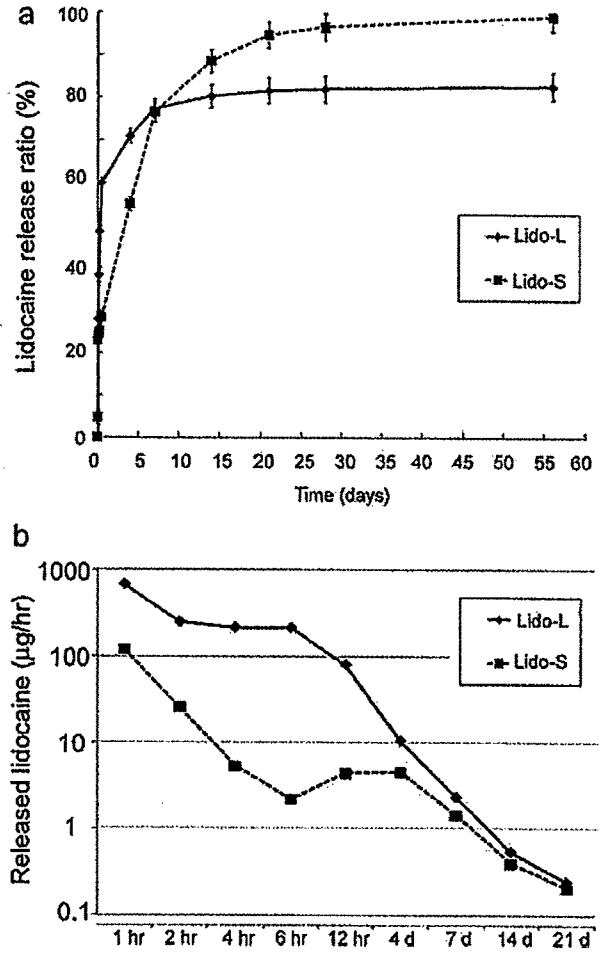

Fig. 3. In vitro-release profiles of lidocaine from Lido-L and Lido-S microparticles. The closed squares show the data for Lido-S microparticles, and the closed diamonds show the data for Lido-L microparticles. (a) Cumulative amounts of lidocaine released from Lido-L or Lido-S microparticles at each time point. The value (%) was calculated as follows: (cumulative amount of lidocaine released)/(total lidocaine content)×100. (b) The amount of lidocaine released per hour at the following time points: 0 to 1 hour, 1 to 2 hours, 2 to 4 hours, 4 to 6 hours, 6 to 12 hours, 12 hours to 4 days, 4 to 7 days, 7 to 14 days, and 14 to 21 days.

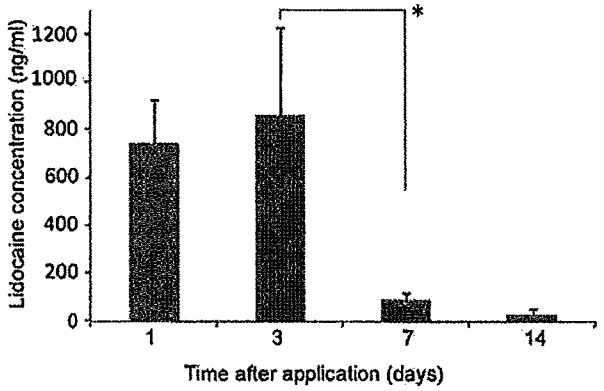

Fig. 4. Lidocaine concentrations in the perilymph following local application of Lido-L microparticles. The bars represent the standard errors. *Indicates a significant difference by analysis of variance with the Tukey-Kramer test.

Interestingly, the animals treated with Lido-L microparticles showed a temporal elevation of the ABR thresholds on day 7 at each frequency, although the lidocaine concentration in the perilymph decreased significantly at this time point. The overall effects of Lido-L microparticle application on the ABR thresholds were statistically significant in comparison with those in animals treated with lidocaine-free particles (4 kHz, $P = .0295$; 8 kHz, $P = .0016$; and 16 kHz, $P = .001$). In the Lido-L microparticle-treated animals, pair-wise comparisons demonstrated significant differences in the ABR thresholds between day 7 and before or day 1 or 14 at 4 and 8 kHz, and between day 7 and before or day 1 at 16 kHz (Fig. 5). By contrast, the animals administered lidocaine-free particles showed no significant differences among the time points. The differences in the ABR thresholds between the Lido-L microparticle-treated and lidocaine-free particle-treated animals were significant on day 7 at all tested frequencies (Fig. 5).

Effects on Vestibular Function

Before testing the Lido-L microparticles, we measured the duration of nystagmus following the local application of lidocaine hydrochloride at various concentrations, and the lidocaine concentration in the perilymph 15 minutes after the local application of 1% or 2% lidocaine hydrochloride, to examine the efficacy of our system for the estimation of nystagmus. The local application of 0.5% lidocaine hydrochloride did not cause nystagmus. However, nystagmus was induced in the animals treated with 1%, 2%, or 4% lidocaine hydrochloride. The latency time was 2 minutes with 1%, 2%, or 4% lidocaine hydrochloride treatment, and nystagmus with horizontal components developed toward the treated side for approximately 10 minutes. The nystagmus then gradually disappeared in the animals treated with 1% lidocaine hydrochloride. In those treated with 2% or 4% lidocaine hydrochloride, paralytic nystagmus with horizontal components developed toward the opposite side and persisted for over 2 hours. RT-HPLC analyses showed that the lidocaine concentrations in the perilymph were 10,708 ± 4,606 ng/mL after the application of 1% lidocaine hydrochloride and 17,384 ± 4,027 ng/mL after the application of 2% lidocaine hydrochloride. These findings confirmed that our system detected the nystagmus induced by lidocaine delivered into the cochlear fluid. We then examined the occurrence of nystagmus following local Lido-L microparticle application in 16 animals. Nystagmus and abnormal behaviors indicating vestibular dysfunction were not observed in any of these guinea pigs.

Inflammatory Responses

No severe inflammatory responses, including effusion or swelling of the mucosa, were identified in either Lido-L microparticle-treated or sham-operated ears. However, inflammatory cells were present in the middle

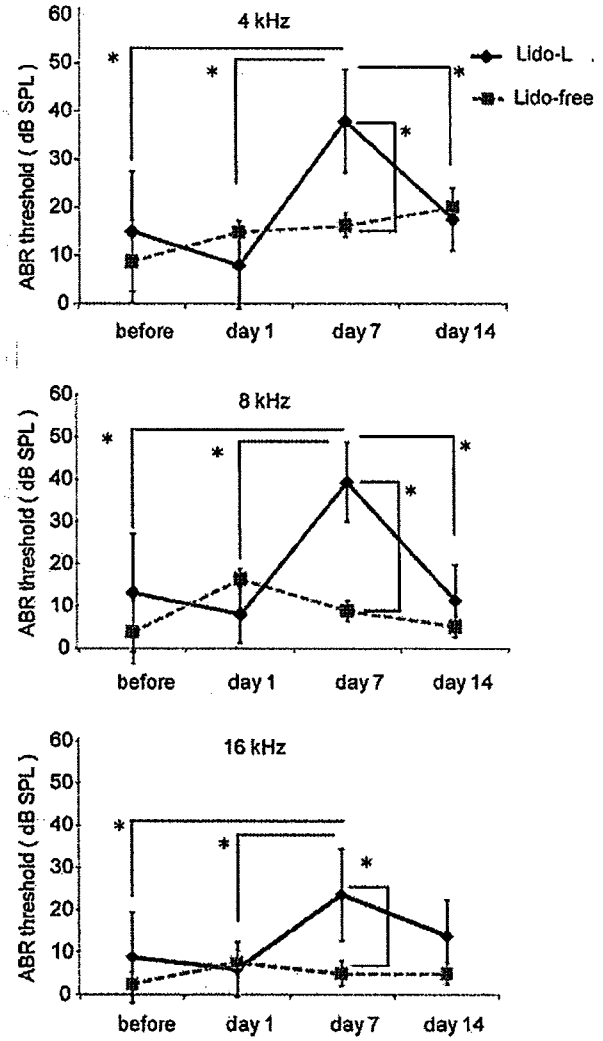

Fig. 5. Auditory brainstem response (ABR) thresholds 4, 8, and 16 kHz before and after local application of Lido-L microparticles or lidocaine-free poly lactic/glycolic acid microparticles. The closed diamonds show the data for Lido-L microparticles (Lido-L), and the closed squares show the data for lidocaine-free particles (Lido-free). *Indicates significant differences according to the Tukey-Kramer test.

ear mucosa of both groups (Fig. 1b, 1c). The numbers of lymphocytes, neutrophils, and plasmacytes in the Lido-L microparticle-treated specimens were 15.3 ± 3.6, 2.8 ± 1.2, and 6.8 ± 1.3, and those in the sham-operated specimens were 16.0 ± 2.8, 4.8 ± 1.1, and 7.3 ± 2.5, respectively. There were no significant differences in the numbers of lymphocytes, neutrophils, or plasmacytes between the Lido-L microparticle-treated and sham-operated specimens.

DISCUSSION

The analyses of the in vitro-release profiles demonstrated that the Lido-L microparticles released lidocaine in a typical sustained-release fashion, whereas the Lido-S microparticles released the majority of the loaded lidocaine during an initial burst. The lidocaine concentrations in the perilymph after the local application of Lido-L microparticles to the guinea pig cochleae demonstrated sustained delivery into the cochlear fluid. High amounts of lidocaine in the perilymph were found a couple of days after the local application, and measurable concentrations were still present after 14 days. These findings demonstrated that Lido-L microparticles were capable of sustained lidocaine delivery into the perilymph after application to the guinea pig RWM.

Local lidocaine application has the advantage of the elimination of systemic side effects compared with systemic lidocaine application. However, local application has side effects including vestibular dysfunction. We examined the occurrence of nystagmus to evaluate the risk of vestibular dysfunction following the local application of Lido-L microparticles. Nystagmus was not observed after the local application of Lido-L microparticles to guinea pigs, although the local application of 2% or 4% lidocaine hydrochloride caused severe nystagmus, as previously reported in rats.[8] The deficit of nystagmus after local application of Lido-L microparticles may be due to a lower concentration of lidocaine in the perilymph than that necessary for anesthetic actions on vestibular peripheral systems. We also examined the inflammatory responses in the middle ear mucosa of guinea pigs following the local application of Lido-L microparticles. No significant infiltration of inflammatory cells was observed in the Lido-L microparticle-treated specimens compared with the sham-operated specimens. Local application using lidocaine-loaded PLGA microparticles, therefore, appeared to be a safe strategy for the sustained delivery of lidocaine into the cochlear fluid.

The present study demonstrated interesting effects of the local application of Lido-L microparticles on hearing. ABR recordings showed a temporal elevation of thresholds on day 7 after application, but no permanent threshold shifts. Histological analyses revealed no significant damage to the middle ear mucosa on day 7 after the local application of Lido-L microparticles, indicating that the observed ABR threshold shifts were not conductive hearing loss. In the ABR recording experiments, lidocaine-free PLGA particles were applied locally to control animals, which showed no significant alterations in the ABR thresholds. The temporal elevation of ABR thresholds observed on day 7 might have been caused by the effects of sustained lidocaine delivery into the cochlear fluid on the auditory pathway. These findings demonstrate that the local application of Lido-L microparticles have certain effects on the auditory system, which can be associated with a beneficial (silencing tinnitus), or an adverse effect (progression of hearing impairment). We should precisely examine the risk for progression of hearing impairment by local application of Lido-L microparticles before clinical application.

The therapeutic effects of lidocaine on the Purkinje fibers in the heart are generally associated with plasma levels of 6 to 25 μM (1.5–6 μg freebase per mL).[9] In general, the suppression of tinnitus by systemic lidocaine application has occurred at equal or lower plasma levels of lidocaine. The present findings demonstrated that the lidocaine concentrations in the perilymph during the initial 3 days after application were maintained at approximately 0.8 μg freebase per mL. We therefore propose that the concentrations of lidocaine in the perilymph after the local application of Lido-L microparticles might be sufficient for tinnitus suppression if the targets are located in the cochlea. Experimental studies have indicated that the spiral ganglion neurons might be one origin of tinnitus in the cochlea.[10] Lidocaine is known to cause the failure of depolarization in neurons by blocking sodium channels, resulting in anesthetic effects. The anesthetic effects of lidocaine on the spiral ganglion neurons require local lidocaine application at a concentration of over 40 mM (1% lidocaine hydrochloride).[11] In the present study, 15 minutes after the local application of 1% lidocaine hydrochloride, the concentration of lidocaine in the perilymph reached 10 μg/mL, which was higher than the maximum concentration of lidocaine following local Lido-L microparticle application. However, a study using ^{14}C-lidocaine demonstrated the accumulation of lidocaine in the cochlear modiolus, where the spiral ganglion neurons are located, after the systemic application of lidocaine.[12] It is therefore possible that locally applied lidocaine accumulates in the spiral ganglion neurons, which could explain the temporal threshold shifts observed on day 7 in the present study. However, lidocaine also affects various types of channel and receptor, including potassium channels and N-methyl-D-aspartic acid (NMDA) receptors.[5] The blocking of potassium currents by lidocaine occurs in the outer hair cells, leading to the reduction of cochlear microphonics.[13] The involvement of NMDA receptors in the generation of peripheral tinnitus has also been indicated in an animal model.[14] Hence, further studies are needed to elucidate the mechanisms of lidocaine action in the cochlea.

CONCLUSION

We produced lidocaine-loaded PLGA microparticles that were capable of the sustained delivery of lidocaine into the cochlea after local application to the RWM. The local application of lidocaine-loaded PLGA microparticles had no significant adverse effects, including vertigo and

otitis media, although the ABR thresholds were temporally elevated. These findings suggest a potential use of lidocaine-loaded PLGA microparticles for the attenuation of peripheral tinnitus. The established animal models of tinnitus[15] could be used to test the efficacy of lidocaine-loaded PLGA microparticles for the attenuation of peripheral tinnitus. In addition, it is also crucial to develop methods for diagnosis of peripheral tinnitus before clinical application.

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