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# Novel in vivo imaging analysis of an inner ear drug delivery system: Drug availability in inner ear following different dose of systemic drug injections

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ABSTRACT

Systemic application of drugs is commonly used in clinical situations. Some of these drugs are ototoxic. Since there are few studies on in vivo monitoring of drug delivery dynamics, the time course or bioavailability of drugs in the inner ear of live animals following systemic drug application remains unknown. For instance, it is unknown whether the volume of a drug delivered systemically correlates with its inner ear pharmacokinetics. We previously established a new in vivo imaging system to monitor drug delivery in live mice. In the present study, we used this system to compare drug concentration in the inner ear over time after systemic drug injections. We used transgenic GFAP-Luc mice that harbor a firefly luciferase gene expression cassette regulated by 12 kb of murine GFAP promoter and human beta-globin intron 2. Luciferin delivered into the inner ear of these mice reacts with luciferase, and the resulting signals are detected in GFAP-expressing cells in the cochlear nerve. Thus, we assessed in the inner ear the intensity and duration of luciferin/luciferase signals after systemic injections of different volumes of luciferin. An IVIS® imaging system was used to observe signals, and these signals were compared to the drug dynamics of luciferin delivered through subcutaneous (sc) injections. The volume of sc-injected drug correlated significantly with photon counts measured in the inner ear. Photons were detected almost immediately after injection, peaking 20 min after injection. Drug concentration did not affect inner ear signals. Luciferin injected systemically appeared in the inner ear between highest and lowest concentration. Drug volume is an important parameter to know if the inner ear requires a higher level of the drug. We observed that it is the volume of a drug—not its concentration—that is the important factor. Indeed, the more volume of a drug injected systemically increased the concentration of that drug in the inner ear. This study provides a better understanding of in vivo drug delivery dynamics measured in the inner ear. Further studies will show whether a high dosage of drug is effective or not.

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## 1. Introduction

Sensorineural hearing loss is mostly caused by inner ear disorders. Inner ear diseases, especially idiopathic sensorineural hearing loss (ISSHL), are generally treated with systemic injections of steroids. A few studies have demonstrated that high-dose steroid treatment significantly and rapidly improves the hearing of patients with ISSHL compared to standard treatments (Aoki et al., 2006; Egli Gallo et al., 2013). Specifically, these studies report that systemic high-dose steroid treatments lead to a higher recovery rate than standard prednisone treatment.

Despite the widespread use of systemic injection of drugs to treat inner ear disorders, it is still unknown whether there is a dose-

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dependent relationship between the amount of drug systemically injected and the amount of drug reaching the inner ear.

It is clear from the above-mentioned studies that drugs applied systemically can affect the inner ear. That is why systemic injections of ototoxic drugs, such as aminoglycoside and cisplatin, also should be monitored carefully to avoid or minimize any side effects to the inner ear. Given this, it is surprising that only a few studies have examined the pharmacodynamics of ototoxic drugs and how this affects the inner ear. Dille et al., for example, found that cumulative cisplatin dose and patients' pre-exposure hearing status were significantly related to being at-risk for a shift in hearing (Dille et al., 2012). This means that ototoxic drugs such as cisplatin also cause inner ear damage or hearing loss in a dose-dependent manner.

In another study, intramuscular injections of kanamycin into C57BL/6JOlaHsd mice induced significant dose-dependent bilateral hearing loss, with a moderate rate of mortality (Murillo-Cuesta et al., 2010). Both of these studies analyzed only drug concentrations in the perilymph.

We recently established a new in vivo imaging system to monitor drug delivery to the spiral ganglion cells of the inner ear in live mice in order to compare drug concentrations over time after systemic injections (Kanzaki et al., 2012). This system utilizes the enzyme luciferase and its ligand, luciferin, which reacts to generate a measurable luminescent product. Unlike methods used in previous studies that measured drug levels in perilymph, our system permits analysis of drug delivery into the inner ear tissues. In the present study, we used this system to determine whether high doses of drugs can be delivered into the inner ear when the drugs are injected systemically.

## 2. Materials and methods

### 2.1. GFAP-Luc mice

Transgenic GFAP-Luc mice that express luciferase were purchased from Xenogen Co., Ltd. These mice harbor a firefly luciferase gene expression cassette that is regulated by 12 kb of the murine GFAP promoter and the human beta-globin intron 2 (Zhu et al., 2004).

Luciferin injected subcutaneously can be detected in the inner ear of these mice as it reacts with luciferase. The resulting signals are detected in GFAP-expressing cells in the cochlear nerve.

All experiments were approved by and carried out in accordance with the Animal Care and Use Committee of Keio University (Permit Number 08020), which is in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA).

### 2.2. Groups

We divided the mice into five groups based on the type of mouse and D-luciferin dose (Table 1). We removed the auricle to facilitate monitoring of luciferin delivery to the cochlea. Different volumes or concentrations of D-luciferin were injected into two types of mice (GFAP-Luc or wild type [WT]) aged 6–8 weeks old (body weight: 26–34 g).

### 2.3. Bioluminescence imaging

An IVIS spectrum and CCD optical macroscopic imaging system (Caliper, Tokyo, Japan) was used for spatiotemporal detection of the luciferase–luciferin reaction. *In vivo* bioluminescent images were captured immediately after subcutaneous (sc) injection of the luciferase substrate, D-(−)-2-(6′-hydroxy-2′-benzothiazolyl) thiazone- 4- carboxylic acid (D-luciferin), with the field of view set at 10 cm. The animals received D-luciferin (dose range: 0.2525–0.7575 mg/g body weight).

Photon count was analyzed between 0 (time of injection) and a minimum of 240 min after sc injection of D-luciferin. Integration time (the device integrated accumulated signals into one picture) was fixed at 5 min for each image. All images were analyzed with Igor (WaveMetrics, Lake Oswego, OR, USA) and Living Image software (Xenogen, Alameda, CA, USA). Optical signal intensity was expressed as flux of photons (photon count), in units of photons/s/cm$^2$/steradian. Each image was displayed as a pseudocolored photon-count image superimposed on a grayscale anatomic image of the inner ear. To quantify the measured light, we defined regions of interest (ROI) in the inner ear and examined all values in that ROI.

We analyzed four parameters: (1) peak photon count, (2) $T_{max}$ (time-to-peak), (3) $T_{1/2}$ (biological half-life), and (4) area under the curve(AUC).

AUC was analyzed using free software ("moment.xls" was downloaded from the Department of Biopharmaceutics and Drug Metabolism, Kyoto University, http://www.pharm.kyoto-u.ac.jp//byoyaku/English/).

### 2.4. Statistics

The test used for pairwise comparisons following a statistically significant one-way ANOVA was the Bonferroni test. For the statistical analyses, we used SPSS 22 software (IBM Corp., Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA).

## 3. Results

Bioimaging demonstrated that luciferase kinetics could be quantitatively evaluated for its time course in the inner ear (Fig. 1). The different peak photon counts for mice receiving different volumes of D-luciferin are shown in Fig. 2. The peak photon counts for mice receiving 3X volume of D-luciferin was significantly different from those receiving 1X volume of D-

**Table 1**
Experimental groups.

| Group | Drug concentration | Drug volume | Total volume of drug (mg/body weight gram) | Mouse | N |
|---|---|---|---|---|---|
| 1 | 1X | 1X | 0.2525 | GFAP-Luc | 4 |
| 2 | 2X | 1X | 0.505 | GFAP-Luc | 5 |
| 3 | 1X | 2X | 0.505 | GFAP-Luc | 5 |
| 4 | 1X | 3X | 0.7575 | GFAP-Luc | 5 |
| 5 | 0 (normal saline) | 1X | 0 | GFAP-Luc | 2 |
| 6 | 1X | 1X | 0.2525 | WT | 3 |