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Transtympanic Delivery of Local Anesthetics for Pain in Acute Otitis Media

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Abstract

Acute otitis media (AOM) commonly causes pain and distress in children. Existing analgesic otological drops have limited effectiveness due to the impermeable nature of the tympanic membrane. We developed a local drug delivery system to provide sustained pain relief in patients with AOM, achieved by applying a single dose of a hydrogel formulation onto the tympanic membrane. Successful drug delivery across intact tympanic membranes was demonstrated using the amino-amide anesthetic, bupivacaine, and a highly potent site 1 sodium channel blocker anesthetic, tetrodotoxin. The chemical permeation enhancers incorporated in the delivery system increased the permeability of the tympanic membrane to the anesthetics considerably. The drug levels measured using a previously developed ex vivo model reflect the potential for highly effective local anesthesia.

Graphical Abstract

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The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.molpharmaceut.8b01235](https://doi.org/10.1021/acs.molpharmaceut.8b01235). FTIR and NMR spectra of P407-PBP (PDF)

Keywords

local anesthesia; chemical permeation enhancers; transtympanic permeation; otitis media; bupivacaine

1. INTRODUCTION

Acute otitis media (AOM) is one of the most common childhood diseases, accounting for over 20 million physician visits each year in the U.S.^{1,2} Recurrence is also common, with one-third of children having six or more episodes of AOM by the age of seven.³ Up to 80% of children with AOM have mild to severe pain during the onset of the infection, of which about 40% have severe pain.^{4,5} The first 24 to 48 h are considered to be the most painful period of AOM, but about 30% of children have pain for 3–7 days.⁶ Consequently, many AOM guidelines recommend the use of analgesics as an essential part of the treatment.⁷

Oral analgesic medications are commonly used,⁸ although it is not clear that they are helpful.⁹ The effectiveness of commercial ototopical products in AOM is also questionable.^{10,11} Nonetheless, local topical treatment of pain in AOM remains desirable, since side effects from systemic drug distribution would be avoided, the pain relief could be faster and more intense during onset, and last longer than with oral analgesia.

We hypothesized that the lack of efficient analgesic effects from ototopical drops was a result of inability to penetrate the tympanic membrane (TM). The outermost layer in the TM, the stratum corneum, is impermeable to virtually all molecules except small and moderately hydrophobic molecules. The stratum corneum barrier can be disrupted by chemically and biologically active molecules and/or physical means.¹² Chemical permeation enhancers (CPEs), in particular, have emerged as an effective means of enhancing small molecule flux across the TM.^{13,14} CPEs can reversibly increase the fluidity of the lipid bilayers in the interstitial space between impermeable corneocytes within the stratum corneum, greatly improving the transdermal delivery of molecules that would otherwise permeate poorly.^{13,14} Thus, a formulation combining CPEs and known anesthetics could enhance drug flux into and across an intact TM and achieve effective analgesia for AOM.

We have previously described a transtympanic drug delivery system that utilizes a hydrogel compound, penta-block copolymer poloxamer 407-polybutylphosphoester (P407-PBP) with three CPEs:^{13,14} sodium dodecyl sulfate (SDS), limonene (LIM), and bupivacaine-hydrochloride (BUP). That combination of CPEs brought ciprofloxacin across an intact TM and treated AOM in a chinchilla animal model successfully.¹⁴ The formulation was administered as a single dose via the ear canal directly on the chinchillas' TM.

P407-PBP was used here because of its robust reverse thermal gelation behavior.¹⁴ It is an easy-to-apply liquid at room temperature and gels quickly and firmly upon contacting the warm TM, holding the antibiotic and CPEs in place (i.e., on the TM). The sustained release and diffusion of drugs into the middle ear can thus be achieved by a single application of the formulation, resulting in a high concentration of drug in the middle ear fluid.¹⁴ Hydrogels have been widely used for sustained-delivery formulations to achieve local analgesia.^{19,20}

Here, we studied the use of this transtympanic drug delivery system to deliver local anesthetics across the TM. We studied bupivacaine, an amphiphilic amino-amide local anesthetic in current clinical use, which we have found has intrinsic activity as a CPE. We also studied tetrodotoxin (TTX), a very hydrophilic compound that blocks the same sodium channel as bupivacaine but at a different site and has ultrapotent local anesthetic activity. Bupivacaine and TTX are known to strongly increase each other's anesthetic effects when given in combination.^{15–17}

2. EXPERIMENTAL SECTION

2.1. Materials.

2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), *n*-butanol, diethyl ether, acetic acid, anhydrous dichloromethane, anhydrous tetrahydrofuran, SDS, LIM, and US pharmaceutical grade BUP and bupivacaine free base (BUP-fb) were used as received from Sigma-Aldrich (St. Louis, MO). US pharmaceutical grade TTX was used as received from Abcam Inc. (Boston, MA). US pharmaceutical grade Kolliphor P407 microprilled (pelletized into microparticles) was received from BASF (Florham Park, NJ).

2.2. Animal Maintenance.

Healthy adult male chinchillas weighing 500 to 650 g were purchased from Ryerson Chinchilla Ranch (Plymouth, OH) and cared for in accordance with protocols approved institutionally and nationally. Experiments were carried out in accordance with the Boston Children's Hospital Animal Use Guidelines and approved by the Animal Care and Use Committee.

2.3. Synthesis of Butoxy-2-oxo-1,3,2-dioxaphospho-lane (BP).

BP was prepared as reported previously.¹⁴ Briefly, BP was synthesized by condensation reaction of COP and *n*-butanol. COP (5.0 g, 35 mmol) in anhydrous THF (50 mL) was added to a stirring solution of *n*-butanol (2.6 g, 35 mmol) and trimethylamine (3.9 g, 39 mmol) in anhydrous THF (100 mL) at 0 °C dropwise. The reaction mixture was stirred in an ice bath for 12 h upon completed addition of COP in THF. Upon complete conversion of COP, the reaction mixture was filtered, and the filtrate was concentrated. The concentrated filtrate was purified by vacuum distillation under reduced vacuum to yield a viscous colorless liquid.

2.4. Synthesis of P407-PBP.

P407-PBP was synthesized as reported previously,¹⁴ by ring-opening polymerization (ROP) of BP with P407 as the macroinitiator in the presence of an organocatalyst, DBU, at –20 °C. ¹⁸ P407 (8.1 g, 0.56 mmol) and BP (1.0 g, 5.6 mmol) in anhydrous dichloromethane (DCM, 0.5 mL) were added to a flame-dried Schlenk flask (10 mL) equipped with a stir bar. The reaction mixture was flushed with nitrogen gas for 5 min while immersed in an ice bath with saturated NaCl solution. A solution of DBU in anhydrous DCM (0.13 g, 0.84 mmol) was added to the stirring solution via a syringe dropwise while maintaining the reaction under nitrogen gas atmosphere. Upon completion of the reaction, an excess amount of acetic acid in DCM was added to the reaction mixture to quench the reaction. The product was purified

by precipitation into ether (three times) and dried under vacuum to obtain a white powder product.

2.5. Hydrogel Formation.

Solutions of 12% (w/v) P407-PBP hydrogel formulations were made by addition of powdered polymers to distilled and deionized water and simple dissolution in a cold room to allow better solubility of P407-PBP. SDS, and/or LIM, and/or BUP, and/or TTX were added to the solution of 12% (w/v) P407-PBP and allowed to dissolve in a cold room for at least 4 h. The TTX hydrogel formulations were made with citrus buffer to enhance TTX solubility.

2.6. In Vitro Release Studies.

The release of BUP or TTX from each formulation was measured using a diffusion system. Transwell membrane inserts (0.4 μm pore size, 1.1 cm^2 area; Costar, Cambridge, MA) and 24-well culture plates were employed as the donor and acceptor chambers, respectively. Each formulation (200 μL) was pipetted directly onto prewarmed filter inserts to obtain a solid hydrogel. Filter inserts (donor compartments) with formed gels were suspended in wells (acceptor compartments) filled with prewarmed phosphate buffered saline (PBS), and the plates then kept in a 37 °C incubator. At each time point (0.5, 1, 2, 6, 12, 24, 48 h), 1 mL aliquots of the PBS receiving media were sampled, and inserts were sequentially moved into a new well with fresh PBS. Aliquots were suspended in 70:30 acetonitrile/PBS to ensure total drug dissolution. Sample aliquots were chromatographically analyzed with high-performance liquid chromatography (HPLC) to determine BUP concentrations (absorption at the wavelength $\lambda = 254 \text{ nm}$) or analyzed with a REAGEN TTX Elisa test kit (Reagen LLC, Collingswood, NJ) to quantify TTX concentrations. Experiments were performed in quadruplicate.

2.7. Ex Vivo Permeation Experiment.

The transtympanic permeation rate of BUP and/or TTX was determined with auditory bullae harvested from healthy chinchillas. Chinchillas were placed under deep general anesthesia by the intramuscular administration of ketamine (30 mg/kg) and xylazine (4 mg/kg) and then euthanized with intracardiac administration of pentobarbital (100 mg/kg). Euthanized animals were decapitated, and the auditory bullae were removed undamaged, with the tympanic ring still attached. Their integrity was assessed by measuring their electrical impedance (indicated by a resistivity $18 \text{ k}\Omega\cdot\text{cm}^2$; a value previously determined¹³) in a setup where TMs were placed horizontally in a 12-well plate with donor solution above and recipient solution below. The same setup was used to measure drug flux, in lieu of a conventional diffusion cell, which would deform or rupture the TM. All formulations were applied into the bullae kept at 37 °C and deposited onto the TMs. The concentration of BUP ranged from 0.5 to 15%, and the volume applied was 200 μL , which translates to 1–30 mg of BUP. The concentration of TTX ranged from 0.02 to 0.32% (solubility limit of TTX), and the volume applied was 200 μL , translating to 0.03 to 0.64 mg of TTX. The BUP and TX concentrations in the receiving chamber were measured at 0.5, 1.0, 2.0, 6.0, 12, 24, and 48 h after the administration of the hydrogel compound. Permeation of BUP and/or TTX across the TM into the receiving chamber was quantified using HPLC or a TTX Elisa kit. Detailed

information regarding TM harvesting, TM electrical resistance measurement, and configuration of the ex vivo permeation experiment can be found in reference 13.

2.8. Histopathology.

Hydrogel formulations containing anesthetics and CPEs were administered to the ear canals of healthy chinchillas. They were euthanized as described above, 24 h to 7 days later. Following sacrifice, the bullae were excised as described above to obtain samples of the TM and the external auditory meatus. Excised tissues were immediately fixed with 10% formalin overnight and then decalcified, embedded in paraffin, sectioned (10 μ m thick), and stained with hematoxylin and eosin. All stained specimens were evaluated by light microscopy in a blinded fashion.

2.9. Statistical Analysis.

For the ex vivo experiments, a sample size of four for each formulation was chosen, which would provide 80% power to detect 50% differences in flux based on power analysis using the nonparametric Friedman test (version 7.0, nQuery Advisor, Statistical Solutions, Saugus, MA). Statistical analysis was conducted using Origin 8 software (version 9.2, SAS Institute, Cary, NC). Data were presented as median (first quartile–third quartile).

2.10. Calculation of Hypothetical Drug Levels in Middle Ear Fluid.

We made the following assumptions in order to calculate the middle ear concentrations of bupivacaine and TTX that would be achieved in vivo:

- (1) The fluid turnover rate is zero in the middle ear of AOM patients (i.e., middle ear fluid is not replenished), because middle ear fluid drainage is impeded by inflammation of the Eustachian tube mucosa in AOM.¹⁹
- (2) Drug concentration changes due to absorption by the surrounding middle ear mucosa, digestion by bacteria and enzymes, etc. are negligible.
- (3) The average volume of the human middle ear is ~0.45 mL.²⁰
- (4) Infinite sink conditions, which were applied during ex vivo experiments where the receiving chamber volume is 3 mL, still hold true for the human middle ear volume of 0.45 mL.

The measured cumulative mass of drug to have crossed the TM at any time point was divided by the volume of the human middle ear (0.45 mL) to provide the concentration that could have been achieved by a given formulation.

3. RESULTS

3.1. Overview and Nomenclature of the Formulation.

The abbreviations used in the Results and Discussion sections are summarized at the end of the text.

Hydrogel formulations were made in aqueous solutions of the penta-block copolymer P407-PBP at 12% (w/v), with or without additional CPEs and with or without the local anesthetics

BUP [0.5 to 15% (w/v); concentrations above 4% (w/v) were suspensions, which were labeled with the subscript _{susp}] and/or TTX [0.02 to 0.32% (w/v)] (Table 1). When CPEs were added, the composition was 1% (w/v) SDS with 2% (w/v) LIM; this combination was referred to as 2CPE. The gels are referred to as $x\%$ BUP_(susp)- $y\%$ TTX-2CPE-[P407-PBP], where x and y are the weight by volume percentage concentrations of BUP and TTX, respectively. P407-PBP (12%) was used throughout this work, as it was easily extruded from a syringe at room temperature and gelled rapidly at body temperature.¹⁴ (The latter property would be important when applying the materials in toddlers who prefer not to stay still. The hydrogel is necessary for the continuous exposure of TMs to CPEs and anesthetics.¹⁴) If a component was absent from a formulation, it was omitted from the nomenclature. Unless specified otherwise, all percentages are weight by volume percent. The formulation containing BUP-fb dissolved in pure LIM was referred to as $x\%$ BUP-fb-LIM, where x was the weight by volume percentage concentration of BUP. Table 1 summarizes the formulations discussed in this paper and their compositions.

P407-PBP was synthesized by ring-opening polymerization, as reported.¹⁴ Nuclear magnetic resonance (NMR) confirmed the presence of the PBP moieties and determined the degree of polymerization of the PBP moieties to be 5 (Figure S1). Fourier transform infrared spectroscopy (FTIR) confirmed the successful synthesis of the penta-*block* copolymer P407-PBP (Figure S1).

3.2. Effect of BUP Concentration on Transtympanic Permeation Rate.

The transtympanic permeation rate of BUP was assessed using a previously reported *ex vivo* method.¹⁴ In brief, drug transport across the TM was studied at 37 °C using auditory bullae excised from healthy chinchillas. Aliquots of 200 μ L of anesthetic formulations (donor solution) were placed on one surface of the TM (see Experimental Section for details), and flux into 3 mL of PBS (recipient solution) was measured over time (Figure 1).

Flux of BUP across the TM from BUP-2CPE-[P407-PBP] formulations was studied in the BUP concentration range of 0.5 to 15% (Figure 1). Note that BUP was only soluble at concentrations up to 2% in water and up to 4% in 12% [P407-PBP] solution. Therefore, the formulations of 7.5% BUP_{susp}-2CPE-[P407-PBP] and 15% BUP_{susp}-2CPE-[P407-PBP] were suspensions of dissolved and solid BUP. BUP flux increased continuously with increasing BUP concentration up to ~7.5%.

At 6 h, BUP permeation across the TM in the presence of 2CPE was about 1.5 μ g (1.1–1.9 μ g) (median with interquartile range in parentheses) for 0.5% BUP-2CPE-[P407-PBP] (Figure 1). Increasing the BUP concentration from 0.5 to 1% improved the transtympanic flux of BUP by about 28-fold, yielding a 6 h BUP cumulative permeation of 42.7 μ g (27.4–71.7 μ g). Further increasing the BUP concentration to 2 or 4% did not yield much improvement in the BUP flux, with 2% BUP-2CPE-[P407-PBP] achieving 51.0 μ g (35.3–68.1 μ g) and 4% BUP-2CPE-[P407-PBP] achieving 48.0 μ g (43.9–51.2 μ g). The suspension, 7.5% BUP_{susp}-2CPE-[P407-PBP], further increased the 6 h BUP cumulative permeation to 141.1 μ g (85.6–168.8 μ g); there was no further increase with 15% BUP_{susp}-2CPE-[P407-PBP] [163.6 μ g (74.3–223.2 μ g)].

At 48 h, increasing the BUP concentration from 0.5 to 1% increased the BUP flux from 27.0 μg (19.4–31.5 μg) to 208.1 μg (127.7–340.8 μg), a 8-fold enhancement (Figure 1B). Further increasing the BUP concentration to 2% yielded a small increase in BUP flux, to 296.4 μg (206.1–395.7 μg). Doubling the BUP concentration again, to 4%, achieved another 2-fold increase in BUP flux, to 671.9 μg (479.4–820.9 μg). The maximum cumulative permeation of BUP was achieved with 7.5% BUP_{susp}-2CPE-[P407-PBP], which resulted in 1251.2 μg (971.5–1471.0 μg) of BUP crossing the TM by 48 h. The quantity of BUP that permeated across the intact TM corresponded to ~8.3% of the total BUP applied on the TM. Increasing the BUP concentration from 7.5 to 15% did not yield any further enhancement of the 48 h permeation.

3.3. Effect of TTX Concentration on Transtympanic Permeation Rate.

Flux of TTX across the TM was evaluated by the same ex vivo method. The concentration of TTX was determined by enzyme-linked immunosorbent assay (ELISA) (see Experimental Section for details). The concentration of TTX in TTX-2CPE-[P407-PBP] was varied from 0.02% (0.5 mM) to 0.32% (10 mM, Figure 2), where 0.32% (10 mM) was the solubility limit of TTX.

At 6 h, transtympanic permeation of TTX increased roughly linearly with the TTX concentration in the formulation (Figure 2B). Increasing TTX concentration from 0.02% (0.5 mM) to 0.16% (5 mM, i.e., 10-fold) resulted in a 6-fold increase of TTX permeability, from 0.2 μg (0.2–0.3 μg) to 1.3 μg (0.9–2.0 μg). Doubling the TTX concentration from 0.16% (5 mM) to 0.32% (10 mM) resulted in another 3-fold increase of TTX permeability, from 1.3 μg (0.9–2.0 μg) to 4.4 μg (3.2–5.1 μg). At 48 h, the linear correlation remained between TTX concentration and transtympanic permeability, where 0.02% TTX-2CPE-[P407-PBP] led to 3.0 μg (2.3–4.4 μg) of cumulative permeation of TTX, and 0.03, 0.16, and 0.32% TTX formulations achieved 3-, 9-, and 16-fold enhancement, respectively.

3.4. Formulations Combining BUP and TTX.

Combining BUP and TTX has been shown to enhance the anesthetic effect dramatically.^{15–17,21} Here, the concentration of BUP in the combined formulation was fixed at 2%. The TTX concentration was kept constant at 0.03% (1 mM), because similar concentrations have been used topically.^{22,23} The transtympanic permeability of BUP and TTX was studied in the ex vivo model described above, from 2%BUP-0.03% TTX-[P407-PBP] and 2%BUP-0.03% TTX-2CPE-[P407-PBP] (Figure 3).

At 6 h, only 4.3 μg (0.6–10.8 μg) of BUP permeated across the TM from 2%BUP-0.3% TTX-[P407-PBP]. Incorporating 2CPE into the formulation led to a 3-fold increase of BUP transtympanic permeation. The enhancement effect of 2CPE on TTX permeation was much greater, 29-fold, from 0.1 μg (0–0.2 μg) to 2.9 μg (1.6–4.5 μg).

At 48 h, the cumulative permeation of BUP achieved by 2% BUP-0.3% TTX-[P407-PBP] was ~80.2 μg (47.7–128.1 μg), ~2.0% of the total applied BUP (Figure 3A); the cumulative permeation of TTX was ~0.9 μg (0.4–1.7 μg), ~1.4% of the total applied TTX (Figure 3B). Incorporating 2CPE increased the transtympanic BUP permeation to 350.2 μg (270.1–452.9 μg), ~8.8% of the total amount (4 mg) of BUP applied on the TM (Figure 3A). During the

same period, 9.2 μg (5.2–14.4 μg) of TTX permeated across the TM, corresponding to 14.3% of the total amount of applied TTX (63.9 μg , Figure 3B). The 2CPE combination increased permeability of BUP 4-fold and that of TTX 10-fold.

3.5. Terpene-Based Anesthetic Formulations.

In all of the preceding sections, bupivacaine hydrochloride (BUP) was used to formulate the anesthetic hydrogel because of its hydrophilicity. Nonetheless, the highest soluble concentration was 4%. Increasing the concentration of SDS and/or LIM (the 2CPE) up to their respective solubility limits of 20 and 10% did not improve BUP solubility in water. BUP solubility in water was not affected by tuning the pH of the formulation in the range of 3 to 9 to alter the proportion of bupivacaine in the salt form (higher at lower pH) and the more hydrophobic free base.

To increase the soluble BUP concentration in the formulation, bupivacaine free base (BUP-fb) was used instead and dissolved in pure LIM. Pure LIM was chosen as the solvent because of its hydrophobicity,²⁴ its proven permeation enhancement effect,^{13,14,25} and its FDA-approved status for topical applications. The solubility limit of BUP-fb was ~10% in pure LIM, the highest soluble bupivacaine concentration we were able to establish thus far.

Using 10%BUP-fb-LIM in the above ex vivo flux model, the cumulative amount of BUP-fb delivered into the middle ear was 63.5 μg (45.3–68.9 μg) after 0.5 h. The middle ear drug level increased 3- and 27-fold after 6 and 48 h (Figure 4). The transtympanic drug permeability achieved by 10%BUP-fb-LIM [1709.8 μg (1600.1–1742.5 μg)] was not statistically significantly different from that of 15%BUP_{susp}-2CPE-[P407-PBP] [1234.5 μg (735.5–1633.8 μg)].

3.6. In Vivo Biocompatibility in the Ear.

Biocompatibility in the ear was tested by treating healthy chinchillas with the anesthetic-containing formulations, followed by histopathology evaluation of the treated ears (see Section 2.8 for experimental details). For the hydrogel formulations, the duration of the treatment was set to 7 days, a typical treatment duration for acute otitis media.² For 10%BUP-fb-LIM, the exposure time was set to 24 h because of the clinically apparent inflammatory reactions by that time (The actual contact time may be less than 24 h due to the fluidity of 10%BUP-fb-LIM.). The inflammatory tissue reactions disappeared after 7 days.

In animals treated with 4%BUP-2CPE-[P407-PBP] or 15% BUP_{susp}-2CPE-[P407-PBP] for 7 days, hematoxylin and eosin (H&E)-stained sections of the TMs looked similar to normal TMs (Figure 5). No inflammation, necrosis, or tissue damage was observed. Moreover, the external auditory meatus of the treated animals looked similar to healthy meatuses in the H&E-stained sections (Figure 6). The sections showed normal epithelium (outermost layer), covering normal adnexal structures/glands, with no inflammation.

Healthy TMs treated with 10% BUP-fb-LIM for 24 h looked similar to the normal TMs (Figure 5). However, a severe acute and chronic inflammatory response was observed in the external auditory meatus of the treated animals (Figure 6). The inflammatory response

consisted of lymphocytes, monocytes, and neutrophils in the epidermis and subepidermal layers of treated animals. In addition, animals that received 10%BUP-fb-LIM exhibited behavioral anomalies such as excessively scratching their treated ears.

4. DISCUSSION

The hydrogel drug delivery system achieved transtympanic delivery of bupivacaine and TTX in a sustained manner. The formulation containing both anesthetics, 2%BUP-0.3%TTX-2CPE-[P407-PBP], delivered $350.2 \pm 102.7 \mu\text{g}$ of BUP and $9.2 \pm 5.2 \mu\text{g}$ of TTX across the TM in 48 h. That corresponds to an average flux of $\sim 7.3 \mu\text{g/h}$ for bupivacaine and $\sim 0.2 \mu\text{g/h}$ for TTX.

The drug concentrations that might occur in humans from the fluxes stated above were calculated as described in the Experimental Section. After 6 h of exposure to 2%BUP-0.3%TTX-2CPE-[P407-PBP], the cumulative flux of drug was such that the bupivacaine concentration in the middle ear could reach 0.03 mg/mL (dividing the cumulative flux of 0.013 mg by 0.45 mL, i.e., 0.09 mM) and the TTX concentration could reach $6.4 \mu\text{g/mL}$ (dividing the cumulative flux of $2.9 \mu\text{g}$ by 0.45 mL, i.e., $20 \mu\text{M}$) TTX. At 48 h, the drug concentrations increased to $\sim 0.8 \text{ mg/mL}$ (dividing the cumulative flux of 0.35 mg by 0.45 mL, i.e., 3 mM) for BUP and $\sim 0.02 \text{ mg/mL}$ (dividing the cumulative flux of $9.2 \mu\text{g}$ by 0.45 mL, i.e., $64 \mu\text{M}$) for TTX.

The concentrations measured in the receiving chamber are the product of drug penetrating throughout the tissue and then exiting, i.e., they reflect the concentrations in the tissue. In considering whether these concentrations would achieve local pain relief, it is useful to first consider what concentrations would result in local anesthesia in tissue. In vitro, bupivacaine inhibits most sodium current with a $K_i = 25 \mu\text{M}$ ²⁶ and reduces the amplitudes of action potentials with a median inhibitory concentration of $180 \mu\text{M}$;²⁷ the corresponding values of TTX are 1–2^{28,29} and 5–6 nM.³⁰ The concentrations in the receiving chamber were all higher than the nano to micromolar concentrations required for nerve block in vitro. For bupivacaine, the concentrations in the receiving chamber were also much higher than the blood (systemic) drug concentrations required to achieve analgesia in animals. A plasma lidocaine concentration of $0.36 \mu\text{g/mL}$ ($1.5 \mu\text{M}$) achieved analgesia in a rat neuropathic pain model;³¹ this was 1.2% of the bupivacaine concentration achieved here at 6 h and 0.05% of the concentration at 48 h. (In addition, bupivacaine is ~ 4 times more potent than lidocaine.³²) The concentrations of TTX achieved at 6 and 48 h here are actually concentrations that achieve nerve block (tens of μM) when used in perineural block.^{33,34}

Human TMs are on average about 10 times thicker than those of chinchillas,³⁵ so the drug concentrations achieved in human middle ears might be lower. While it is possible that higher or multiple doses may therefore be required in humans, the drug concentrations achieved with the chinchilla TMs were as much as 1000 times greater than the effectively analgesic concentrations. Moreover, flux is likely to be even greater in inflamed TMs than in the pristine TMs used here, even though the TMs also become much thicker.¹⁴ That greater drug flux could markedly enhance drug levels in the middle ear, enhancing efficacy. Thus, the permeation results provided here underestimated the flux to be expected in animals with

AOM. Therefore, even with the thicker human TMs, the drug concentrations in the middle ear are likely to be effective.

Moreover, local anesthetic efficacy could be greatly enhanced were bupivacaine and TTX to be codelivered.^{15–17} Conventional amino-amide or amino-ester local anesthetics such as bupivacaine are known to have marked synergy with compounds such as TTX, which block the same sodium channel at a different site termed site 1 on the axonal surface. Concentrations of either compound that would be relatively ineffective independently can become effective in combination. Moreover, CPEs are known to enhance the local anesthetic effect of TTX, presumably by enhancing penetration to the axonal surface.^{36–38}

The effectiveness of ear drops containing anesthetics such as lidocaine is controversial and is short-lived;³⁹ this poor performance is likely a result of the well-known barrier function of the TM.¹² We were able to overcome the permeation barrier and deliver therapeutic levels of bupivacaine and TTX across intact TMs. In vivo, the hydrogel would also extend the effect over a prolonged period, which would likely cover the time frame within which otalgia is at its worst. This would likely be even more effective with dual delivery of conventional local anesthetics and site 1 sodium channel blockers.^{15–17,33}

Although CPEs increased the transtympanic flux of both BUP and TTX, the effect on the flux of TTX (a 10-fold increase at 48 h) was much greater than that on BUP (a 4-fold increase at 48 h). This pattern was reminiscent of the effect of CPEs coinjected with those compounds at the sciatic nerve:³⁶ nerve blockade by TTX was markedly enhanced by CPEs, while that from BUP was not. We had postulated that the reason for this difference was that TTX, being very hydrophilic, had great difficulty penetrating biological barriers and so would benefit from the CPEs. BUP, being amphiphilic, would have less trouble penetrating biological barriers, and so would benefit less from the CPEs.

Although 10%BUP-fb-LIM had a greater dissolved drug concentration than the hydrogel formulations, the transtympanic permeation of BUP was similar. 10%BUP-fb-LIM achieved a BUP concentration of ~0.4 mg/mL (1.2 mM) in the middle ear at 6 h after administration. 10%BUP-fb-LIM caused a severe inflammatory response in the meatus, which could be a result of the high LIM concentration or the high free bupivacaine concentration in the formulation. The inflammatory response was not seen in the TM, presumably because in the absence of the hydrogel, the 10%BUP-fb-LIM flowed off of the TM into the auditory canal once the animals woke up.

The concentration of TTX in 2%BUP-0.3%TTX-2CPE-[P407-PBP] was higher than concentrations that have been injected near nerves to achieve peripheral nerve blockade.^{22,23,40,41} However, here the TTX was applied outside the body; the concentration used was comparable to those used in other topical applications, such as on the cornea.^{22,23} The concentrations to which nerves would be exposed are likely to be much closer to those measured in transtympanic flux, which were much lower and known to be safe in nerve.^{22,23,40,41}

It was somewhat surprising that the hydrogels containing suspensions of bupivacaine, such as 7.5%BUP_{susp}-2CPE-[P407-PBP], increased the transtympanic permeation of bupivacaine

by 2-fold at 48 h compared to hydrogel solutions such as 4% BUP-2CPE-[P407-PBP], since the concentrations of bupivacaine in solution were presumably the same. It is possible that the drug in suspension acted as a drug reservoir replenishing the concentration of free drug on the TM surface as it was depleted by flux.

We have previously shown, using a similar hydrogel delivery system, that transtympanic drug delivery results in no detectable systemic (blood) distribution of the antibiotic ciprofloxacin.^{14,42} Presumably, transtympanic delivery of bupivacaine and TTX would also not result in systemic drug distribution and so would obviate the side effects of the anesthetics. This treatment would also obviate the need for systemic (oral) analgesics and their potential side effects.

The thermosensitive hydrogel was designed to provide sustained pain relief and enable easy administration. The hydrogel formulation is a solution under room temperature for administration through the ear canal like other regular ear drops; the formulation gels quickly in situ upon contacting the warm TM. Only a single application is required to maintain local anesthesia over prolonged periods, which is beneficial because multidose regimens can cause poor compliance among uncooperative young patients.

5. CONCLUSIONS

A local drug delivery system was developed to provide sustained pain relief from a single application in patients with AOM. A commonly used amino-amide anesthetic, bupivacaine, was successfully delivered across intact TMs, as was a highly potent site 1 sodium channel blocker anesthetic, TTX. The chemical permeation enhancers incorporated in the hydrogel system considerably increased the permeability of BUP and TTX across the TM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

BUP	bupivacaine
BUP-fb	bupivacaine free base
CPEs	chemical permeation enhancers
LIM	limonene
P407-PBP	poloxamer 407-polybutylphosphoester
SDS	sodium dodecyl sulfate

Susp	suspension
TTX	tetrodotoxin
TMs	tympanic membranes

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Figure 1.

Cumulative permeation of bupivacaine hydrochloride (BUP) across the tympanic membrane from formulations containing 2CPE-[P407-PBP]. (A) Time course of cumulative permeation of BUP achieved by BUP-2CPE-[P407-PBP] with different BUP concentrations over 48 h. BUP was not soluble in 2CPE-[P407-PBP] beyond 4%. Therefore, the formulations, 7.5% BUP_{susp}-2CPE-[P407-PBP] and 15% BUP_{susp}-2CPE-[P407-PBP] were suspensions, which is indicated by † in the plot. Arrows indicate data graphed in panel B. (B) Effect of bupivacaine concentration on cumulative permeation across the TM at 6 and 48 h, derived from data denoted by arrows in panel A. Data are medians $\pm$ interquartile ranges ($n=4$).

Figure 2.

Cumulative permeation of tetrodotoxin (TTX) across the tympanic membrane from formulations containing 2CPE-[P407-PBP]. (A) Transtympanic permeation of TTX from formulations containing 0.02, 0.03, 0.16, and 0.32% TTX, which corresponds to 0.5, 1, 5, and 10 mM TTX, over 48 h. (B) The dependence of TTX permeation on the drug concentration in the hydrogel formulations. Data are medians $\pm$ interquartile ranges ($n = 4$).

Figure 3. Cumulative ex vivo permeation of (A) BUP and (B) TTX across the tympanic membrane from formulations containing both compounds and [P407-PBP]. Data are medians $\pm$ interquartile ranges ($n=4$).

Figure 4. Cumulative permeation of bupivacaine free base and BUP across the tympanic membrane. BUP was not soluble in 2CPE-[P407-PBP] beyond 4%. Therefore, 15% BUP_{susp}-2CPE-[P407-PBP] was a suspension, which is indicated by † in the plot. Data are medians $\pm$ interquartile ranges ($n = 4$).

Figure 5. Representative H&E-stained sections of TMs treated under different conditions. Scale bar represents 12 μm .

Figure 6.

Representative H&E-stained sections of the untreated external auditory meatus, of the external auditory meatus processed 24 h after exposure to 10% [bupivacaine free base]-LIM, and of the external auditory meatus treated with 4% BUP-2CPE-[P407-PBP] or 15% BUP-2CPE-[P407-PBP] for 7 days. Scale bar represents 50 μm . Inset: Enlarged image highlighting inflammatory cells. Black arrow points to a neutrophil; white arrow with black outline points to a lymphocyte. Scale bar within the inset represents 10 μm . external auditory meatus of the treated animals (Figure 6). The inflammatory response consisted of lymphocytes, monocytes, and neutrophils in the epidermis and subepidermal layers of treated animals. In addition, animals that received 10% BUP-fb-LIM exhibited behavioral anomalies such as excessively scratching their treated ears.

Table 1.Formulations and Their Compositions^a

formulations	P407-PBP	BUP	BUP-fb	TTX	SDS	LIM
Figure 1						
0.5%BUP-2CPE-[P407-PBP]	12	0.5	0	0	1	2
1%BUP-2CPE-[P407-PBP]	12	1	0	0	1	2
2%BUP-2CPE-[P407-PBP]	12	2	0	0	1	2
4%BUP-2CPE-[P407-PBP]	12	4	0	0	1	2
7.5%BUP _{susp} -2CPE-[P407-PBP]	12	7.5	0	0	1	2
15%BUP _{susp} -2CPE-[P407-PBP]	12	15	0	0	1	2
Figure 2						
0.02%TTX-2CPE-[P407-PBP]	12	0	0	0.02	1	2
0.03%TTX-2CPE-[P407-PBP]	12	0	0	0.03	1	2
0.16%TTX-2CPE-[P407-PBP]	12	0	0	0.16	1	2
0.32%TTX-2CPE-[P407-PBP]	12	0	0	0.32	1	2
Figure 3						
2%BUP-0.03%TTX-[P407-PBP]	12	2	0	0.03	0	0
2%BUP-0.03%TTX-2CPE-[P407-PBP]	12	2	0	0.03	1	2
Figure 4						
10%BUP-fb-LIM	0	0	10	0	0	84
15%BUP _{susp} -2CPE-[P407-PBP]	12	15	0	0	1	2
Figures 5 and 6						
10%BUP-fb-LIM	0	0	10	0	0	84
4%BUP-2CPE-[P407-PBP]	12	4	0	0	1	2
15%BUP _{susp} -2CPE-[P407-PBP]	12	15	0	0	1	2

^aUnless specified otherwise, all percentages are weight by volume percent.