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Controlled release of dexamethasone from fibrin sealant for intratympanic administration in inner ear therapy

Jing Zou [a,b,*]

[a] Department of Otolaryngology-Head and Neck Surgery, Changhai Hospital, Second Military Medical University, Shanghai, 200433, China
[b] Faculty of Information Technology and Communication Sciences, Tampere University, Tampere, Finland

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ABSTRACT

The aim of the present work was to show the sustainability of fibrin sealant in releasing dexamethasone and adjust the protocol for clinical application of the novel method in the treatment of Meniere's disease (MD) and sudden sensorineural hearing loss (SSHL). Gelation occurred shortly after mixing dexamethasone-containing fibrinogen with thrombin. Dexamethasone was constantly released for at least 16 d at a stable level after 7 d in protocol 1 (low-dose), while it was robustly released within 4 d and slowed afterward until 10 d in protocol 2 (high-dose). There were significant differences among the time points in Protocol 2 ($p < 0.01$, ANOVA), and the exponential model with the formula $y = 15.299 * e^{-0.483 * t}$ fits the association. The estimated concentration of dexamethasone released on 7 d in protocol 2 was slightly lower than that observed in protocol 1. The fibrin sealant is capable of constantly releasing dexamethasone with adjustable dynamics. Targeted and minimally invasive administration of the material can be achieved in the clinic by sequential injections of the fluids using a soft-tipped catheter.

1. Introduction

Intratympanic injection of dexamethasone has been applied globally as an alternative treatment for Meniere's disease (MD) and sudden sensorineural hearing loss (SSHL) since the first induction in 1991 (Itoh and Sakata, 1991; Silverstein et al., 1996). Sustained delivery of therapeutics may induce a better outcome of diseases than conventional intratympanic injection due to the ability to maintain concentrations in the inner ear and consequently the bioavailability of therapeutics. Sustainable drug delivery is also beneficial by avoiding frequently repeated injections. Various materials have been tested in animals and determined to have potential clinical applications in inner ear therapy (Le et al., 2023; Martin-Saldana et al., 2018; Yu et al., 2016; Zou et al., 2008, 2014). Rod-shaped poly (D,L-lactide-co-glycolide) (PLGA) polymer containing dexamethasone was implanted into the round window niche of patients with SSHL and resulted in excellent hearing outcomes (Plontke et al., 2014). However, middle ear surgery is needed to perform such implantation. A novel material of sustained release dexamethasone formulation, OTO-104, has also been tested in clinical trials, and the final efficacy needs further confirmation (Lambert et al., 2012; Phillips et al., 2023). Currently, OTO-104 has not yet been approved for clinical application and is clinically unavailable. Alternatively, fibrin sealant has been extensively applied in clinical practice and is an excellent candidate material attributed to the capability of controlled release of neurotrophin-3 for up to 28 d (Li et al., 2016).

I chose dexamethasone instead of methylprednisolone because it was reported that methylprednisolone may hydrolyze in a temperature-dependent manner and remain stable for only 24 h at 22 °C (Nahata et al., 1994). Therefore, methylprednisolone may degrade much faster in the middle ear cavity when the temperature is 36 °C and is unsuitable for constant delivery. I have developed the protocol of controlled release of dexamethasone mixed with fibrin sealant for intratympanic administration and have been treating patients with SSHL since 2019 March 17 and MD since 2019 April 11 in a total number of 348 cases (SSHL 125 cases, MD 223 cases) until 2023 May 24. To show the sustainability of the fibrin sealant in releasing dexamethasone and adjust the protocol for clinical application of the novel method in the treatment of MD and SSHL, I report the procedure as follows.

Abbreviations: ANOVA, analysis of variance; HPLC, high-performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; MD, Meniere's disease; PLGA, poly (D,L-lactide-co-glycolide; SSHL, sudden sensorineural hearing loss.

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* Department of Otolaryngology-Head and Neck Surgery, Changhai Hospital, Second Military Medical University, Changhai Road #168, Shanghai, 200433, China.
E-mail address: Jing.Zou@tuni.fi.

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Table 1
Design of analysis of samples released from fibrin sealant with different loading protocols.

Protocol	Dex loading	Sampling time points (day)								Analysis
1	12.5 mg/mL	4[a]	7	10	13	16				HPLC
2	33.3 mg/mL	1	2	3	4	5	6	8	10	LC–MS

Dex: dexamethasone; HPLC: high-performance liquid chromatography. LC–MS: liquid chromatography–mass spectrometry.
[a] Samples at the time point were not analyzed.

2. Materials and methods

2.1. Materials

Fibrin Sealant (Human) (FIBINGLUEAAS®) was purchased from Shanghai RAAS Blood Products Co., Ltd. (Shanghai, China). Dexamethasone (5 mg) was purchased from Ma An Shan Fengyuan Pharmaceutical CO., LTD (Anhui Province, China). A soft tip microcatheter (ZJ-XWCXG-RT-180) was donated by Shijiazhuang Zouji Medical Equipment Limited Science & Technology Co., Ltd. (Shijiazhuang, China) and was made based on patent no. 201721665246.6.

2.2. Delivery protocols

Due to the limited space of the middle ear cavity and the loading capacity of the drug carrier, it is always a challenge to optimize the loading concentration and release dynamics of dexamethasone. First (protocol 1), I prepared the solutions of fibrin sealant components according to the manual of the manufacturer and mixed dexamethasone (50 mg/mL) with fibrinogen solution at a ratio of 1:1 (v/v), and the final concentration of dexamethasone was 12.5 mg/mL. Protocol 2 was developed on June 11, 2020, and was able to carry dexamethasone at concentrations as high as 33.3 mg/mL.

Protocol 1: The lyophilized fibrinogen (human) was reconstituted with 2 mL sterile water, and the lyophilized thrombin (human) was reconstituted with 2 mL calcium chloride solution and then warmed at 37 °C for approximately 5 min with low-speed constant shaking. Then, 0.1 mL sterile water was injected into the vial of dexamethasone to fully resolve the product by shaking. Next, 0.1 mL of fibrinogen solution was injected into the vial, followed by injection of 0.2 mL of thrombin solution. Therefore, the original concentration of dexamethasone in the gel was 12.5 mg/mL. To mimic the clinical application, 0.2 mL of a mixture of fibrinogen and dexamethasone solution and 0.2 mL of thrombin solution were withdrawn separately with 1.0 mL syringes. The steel needle on the syringe was replaced by the abovementioned soft tip microcatheter, which has a tiny soft tip and is suitable for targeted intratympanic drug delivery (Zou et al., 2018, 2019). The two solutions were separately injected into a plastic tray to observe the gelation.

Protocol 2: The lyophilized fibrinogen (human) was reconstituted with 2 mL sterile water, and the lyophilized thrombin (human) was reconstituted with 1 mL calcium chloride solution and then warmed as mentioned above. Sterile water (0.05 mL) was injected into each vial of dexamethasone to fully resolve the product as mentioned above. Then, 0.05 mL of fibrinogen solution was injected into each vial, followed by the injection of 0.05 mL of thrombin solution. Finally, the concentration of dexamethasone in the gel was 33.3 mg/mL.

2.3. Dynamic release of dexamethasone from fibrin sealant

For protocol 1, 0.2 mL of saline was added to each vial, and sampling was attempted on day 2but failed. Therefore, an additional 0.2 mL of saline was injected, and the first sample was taken on day 4; the others were taken on days 7, 10, 13, and 16, followed by the injection of 0.2 mL of saline postsampling at each time point. Six replicates were prepared for each time point. The samples from 4 d were discarded, and only the samples from 7 to 16 d were prepared for quantification using high-performance liquid chromatography (HPLC) (Table 1).

For protocol 2, 0.15 mL saline was added to each vial, and sampling was started on 1 d and continued on 2, 3, 4, 5, 6, 8, and 10 d, followed by injection of 0.15 mL saline postsampling at each time point. Six replicates were prepared for each time point. The samples taken at each time point were processed for quantification using liquid chromatography–mass spectrometry (LC–MS) (Table 1).

2.4. HPLC

The dexamethasone powder was recovered with pure water to prepare a standard solution of 10 mg/mL and serially diluted to the following concentrations for measurements in creating the standard curve: 2.5000, 1.2500, 0.6250, 0.3125, and 0.1563 mg/mL. Three repeats were prepared for each concentration. A 100 μL sample released from the fibrin sealant was diluted with 100 μL of pure water. Standard solutions (200 μL) and diluted samples were filtered through a polytetrafluoroethylene membrane. Five microliters of the filtered final solutions were added to a chromatographic column (Diamonsil Plus-C18B, 5 μm, 250*4.6 mm, Beijing Dikema Technology Co, Ltd., Shanghai, China) in combination with HPLC (1260 Infinity II, Agilent Technologies, Shanghai China). The column temperature was 40 °C, and acetonitrile/water/trifluoroacetic acid at a ratio of 40/60/0.1 (v/v%) was selected as the mobile phase at a flow rate of 1.0 mL/min. The analyte was monitored spectrophotometrically at 242 nm.

2.5. LC–MS

The dexamethasone powder was recovered with acetonitrile/pure water at a ratio of 75/25 (v/v%) to prepare a standard solution of 10 mg/mL and diluted to the following serial concentrations for measurements in creating the standard curve: 4.61, 1.00, 0.45, 0.10, 0.05, and 0.01 mg/mL. Two repeats were prepared for each concentration. Acetonitrile (100 μL) was added to 100 μL samples released from fibrin sealant and stored at 4 °C for 2 h to sediment proteins. After centrifugation at 15000×*g* for 10 min, 100 μL of supernatant was withdrawn and diluted with 100 μL of pure water. Two microliters of the prepared standard solutions and samples were analyzed using LC–MS (Ultimate 3000, Thermo Fisher Scientific, Germering, Germany) in combination with a chromatographic column (Agilent Zorbax-C18, 5 μm 100A, 150*4.6 mm, Agilent Technologies, Inc., California, USA). The column temperature was 25 °C, and formalin/acetonitrile at a ratio of 60/40 (v/v%) was selected as the mobile phase at a flow rate of 0.5 mL/min. The analyte was monitored spectrophotometrically at 240 nm.

2.6. Data analysis and statistics

The concentrations of dexamethasone measured at each time point in protocol 1 were divided by 3 to transform the value to a single-day release. Concentrations of dexamethasone measured at time points of 8 and 10 d in protocol 2 were divided by 2 to transform the value to single-day release. One-way analysis of variance (ANOVA) was performed to compare the differences in dexamethasone release at different time points. Regression analysis was applied to estimate the time-dependent release of dexamethasone. $P < 0.05$ was considered statistically significant.

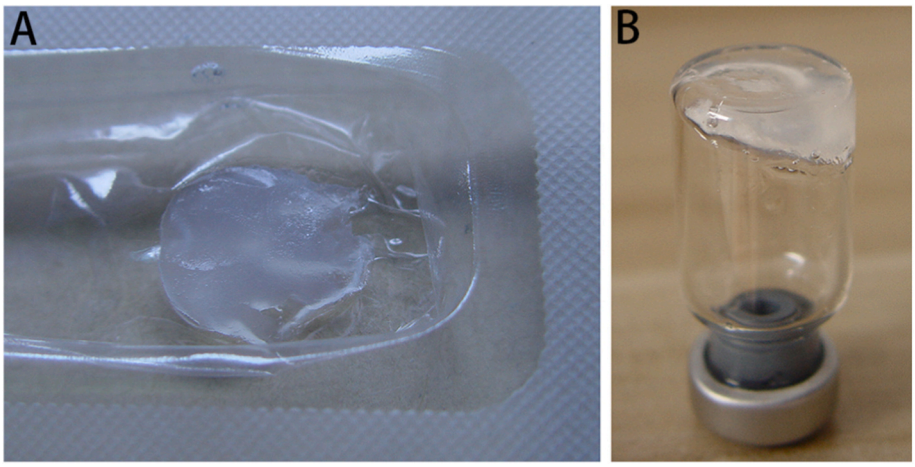

Fig. 1. Illustration of gelation observed in preparations of fibrin sealant loaded with different doses of dexamethasone. Gelation occurred in both protocol 1 (A) and protocol 2 (B).

3. Results

Gelation was observed to occur in a couple of minutes after mixing dexamethasone-containing fibrinogen with thrombin in both protocols (Fig. 1). In protocol 1, the gel of fibrin sealant remained intact after the final sample collection on 16 d. However, in protocol 2, two of the 6 gels started to detach from the bottom of the vial at 8 d, and all gels were fully dissolved on 10 d; this indicated that the release time of fibrin sealant loaded with a lower concentration of dexamethasone (protocol 1) was longer than that loaded with a higher concentration of dexamethasone (protocol 2).

Dynamic release in protocol 1 demonstrated that dexamethasone was detectable for at least 16 days, and the differences in dexamethasone concentrations released from 7 to 16 d were insignificant ($p > 0.05$, ANOVA) (Fig. 2A). In protocol 2, there was a robust release of dexamethasone within 4 d that slowed afterward, and the differences at various time points were significant ($p < 0.01$, ANOVA) (Fig. 2B). The regression showed that the exponential model with the formula $y = 15.299 * e^{-0.483 *t}$ fits the association (Fig. 2C). The average released cargo was 8.9 mg/mL on 1 d, 3.7 mg/mL on 3 d, 1.4 mg/mL on 5 d, and 0.9 mg/mL on 6 d. According to the formula, the estimated concentration on 7 d was 0.5 mg/mL, which was slightly lower than that observed in protocol 1 at the same time point (0.6 mg/mL).

4. Discussion

The current study demonstrated that gelation occurred shortly after mixing dexamethasone-containing fibrinogen with thrombin in both protocols, which proved that adding the therapeutics into a component of the sealant did not prevent the coagulation process. Although the results obtained in the *in vitro* study might be different from those obtained in the *in vivo* study, it is true that the gel of fibrin sealant in protocol 1 dissociated slower than the gel in protocol 2. However, a robust release of dexamethasone at high concentrations was introduced within 4 days in protocol 2. The average released cargo on 1 d was 8.9 mg/mL, which was just below the high dose (12 mg/mL) of reported conventional intratympanic injection, which achieved vertigo control in 91% of MD patients (Boleas-Aguirre et al., 2008). The average amount of dexamethasone released on 3 d was 3.7 mg/mL, which was slightly lower than the dose of conventional intratympanic injection (4 mg/mL) in another report that showed satisfactory control of vertigo in 92% of patients with MD (Weckel et al., 2018). The concentration in protocol 2 remained at an average level as high as 0.9 mg/mL on 6 d, although the estimated concentration on 7 d was slightly lower than that observed in protocol 1.

In the treatment of patients with MD, injections were repeated in 3–5 days (Ren et al., 2015; Weckel et al., 2018). In the treatment of SSHL, intratympanic dexamethasone (4 mg/mL) was reported to be at least as effective as intravenous dexamethasone for SSHL patients with diabetes when it was delivered for 8 sequential days (Kakehata et al., 2006). The amount of dexamethasone released in protocol 2 on earlier days was even higher than that in the conventional procedure for the treatment of SSHL, although it was slightly lower on 3 d than that in the reported conventional injection. Therefore, protocol 2 might be more beneficial for patients with both MD and SSHL than protocol 1. The outcome of treatments for patients with MD and SSHL using these protocols will be reported in a separate study.

The obvious advantage of the current protocols over the conventional method is that the concentration of therapeutic remained in the inner ear, and a single delivery was performed that avoids the frequently repeated injections in the conventional protocol for patients with both MD and SSHL (Boleas-Aguirre et al., 2008; Ren et al., 2015; Weckel et al., 2018). In an animal study on transgenic GFAP-luc mice, conventional intratympanic injection of luciferin induced a peak in the inner ear that dropped to a level even lower than that after intravenous injection after 30 min (Kanzaki et al., 2012). That the patients only need to avoid swallowing for 5 min in the current protocols is an additional advantage to the conventional protocol in which the patients were instructed to avoid swallowing for 30 min, which is a major challenge (Boleas-Aguirre et al., 2008; Kakehata et al., 2006).

There were limitations in the current study in that data were only collected after 7 d of release in protocol 1 due to technical difficulty, and the measurement methods were different between protocols 1 and 2 due to the different periods of the analysis. However, these limitations do not influence the sustainable release of dexamethasone from the fibrin sealant.

5. Conclusion

The fibrin sealant is capable of constantly releasing dexamethasone with adjustable dynamics. The gel in protocol 2 carrying high-dose dexamethasone introduced a robust release at high concentrations within 4 days but lasted for only 10 d. The gel in protocol 1 carrying low-dose dexamethasone introduced a slow release and lasted for more than 16 d. Minimally invasive targeted administration of the material can be realized in the clinic by sequential injection of the fluids using a soft-tipped catheter. The potential difference in the outcome of treatment needs further clinical study.

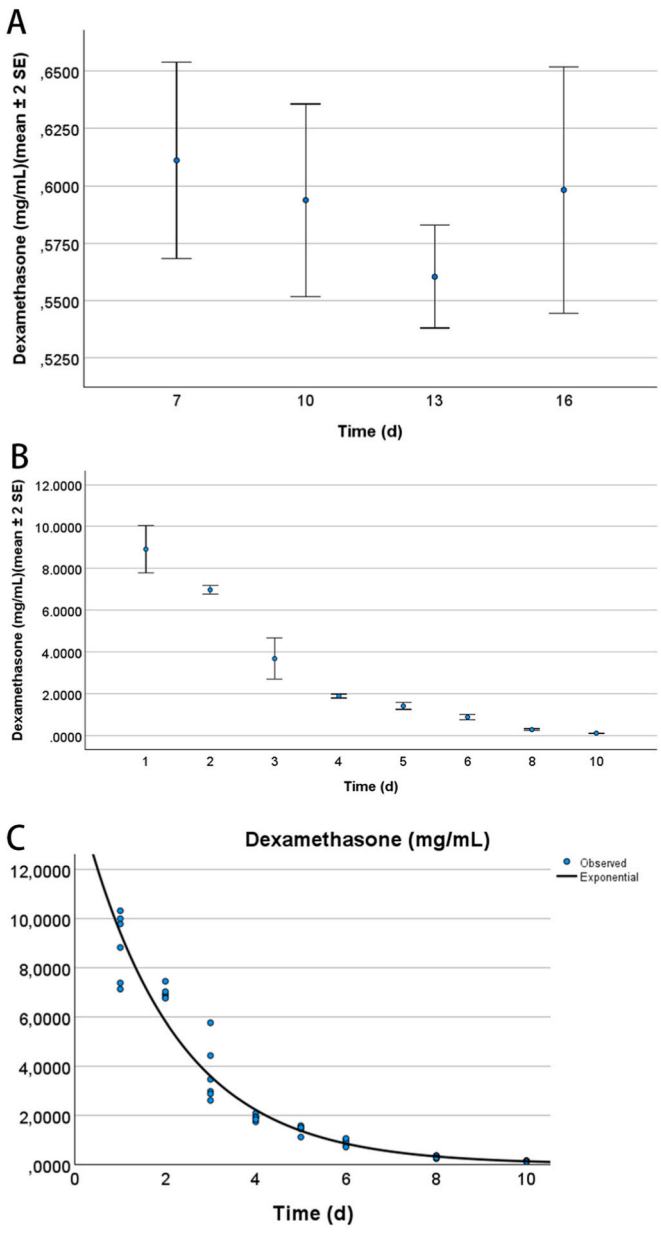

Fig. 2. Dynamics of dexamethasone released from preparations of fibrin sealant loaded with different doses of dexamethasone. The differences in the release dose from 7 to 16 d were insignificant in protocol 1 (A). The differences in the release dose at different time points were significant in protocol 2 (B), which followed an exponential Model (C).

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Declaration of conflicting interest

Jing Zou is the holder of the patent (no. 201721665246.6).

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