Dexamethasone Degradation During *In Vitro* Release from an Intravitreal Implant

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Abstract

Purpose: It has been observed that dexamethasone undergoes degradation after it is released from implants into the aqueous medium during in vitro release testing. In order to obtain correct in vitro release profile, degradation of dexamethasone needs to be properly monitored and measured. The purpose of this study was to develop an LC-MS/MS method to monitor and measure major degradation products of dexamethasone in aqueous medium using both plain dexamethasone solution and release samples obtained from dexamethasone-loaded implants.

Methods: Dexamethasone and dexamethasone-loaded implants made using poly(lactide-co-glycolide) (PLGA) were incubated in PBS buffer (pH 7.4) at 37 and 45 °C, with or without light exposure. Plain dexamethasone solution was also incubated at room temperature. Samples were collected over several days, spiked with d_4-dexamethasone as internal standard, and the concentrations of dexamethasone and thirteen dexamethasone degradation products were analyzed using a QTRAP® 4500 LC-MS/MS.

Results: The mass spectrometer was operated in the MRM mode set to look for the product ions that resulted from the characteristic loss of fluorine from each precursor ion. Using this method, we could detect dexamethasone degradation products in the incubation medium for the implants as well as plain drug. The extent of degradation increased with the study duration as well as light exposure and elevation of temperature. Dexamethasone incubated in PBS buffer at 37 °C, exposed to ambient light was found to have a half-life of about 10 days, while dexamethasone at 45 °C in the absence of light had a half-life of about 7 days. A mathematical model was developed to estimate drug release after accounting for drug degradation.

Conclusions: Dexamethasone, when incubated in aqueous media, produces degradation products in a time, temperature, and light exposure dependent manner. Degradation products were also detected in the in vitro release samples obtained from dexamethasone-loaded PLGA implants. The analytical method developed for monitoring dexamethasone degradation products can potentially be used for assessing the stability of dexamethasone during manufacturing, storage, and use of various dosage forms.

Introduction

Intravitreally administered dexamethasone loaded poly(lactide-co-glycolide) (PLGA) implants are currently indicated for treating macular edema following branch retinal vein occlusion (BRVO) or central retinal vein occlusion (CRVO), non-infectious uveitis affecting the posterior segment of the eye, and diabetic macular edema. Dexamethasone is a fluorinated corticosteroid with anti-inflammatory and immunosuppressive activities. The purpose of this study was to develop an LC-MS method to quantify dexamethasone degradation products in PBS (pH 7.4) and further characterize dexamethasone degradation as a function of temperature and light and during an in vitro release study from a laboratory made PLGA implant of dexamethsone.

Methods

Instrumentation and analysis of dexamethasone and degradation products: : A Shimdazu Prominence HPLC coupled to an AB Sciex QTrap 4500 mass spectrometer was used to develop an analytical method for dexamethasone and its degradation products.

Implant Preparation: A HAAKE MiniCTW Hot Melt Extruder (Thermo Scientific) was used to prepare PLGA implants containing 20% dexamethasone by weight, with a diameter of about 0.4 mm and a weight of about 1 mg.

Dexamethasone degradation and implant release studies: Dexamethasone (0.2 mg) was incubated in PBS (pH 7.4) at 25, 37 or 45 °C (dark). Dexamethasone as well as dexamethasone-PLGA implant were incubated at 37 °C in light as well as dark. An accelerated release study was also performed for the implants at 45 °C (dark only). At predetermined time points, 1 mL of each solution was removed for LC-MS analysis and replaced with fresh buffer. Aliquots from each time point were spiked with d_4-dexamethasone and transferred to well plates for LC-MS/MS analysis.

HPLC-ESI$^+$-MS/MS analysis of dexamethasone: Incubated dexamethasone samples were initially analyzed in the neutral loss mode of the mass spectrometer. The quadrupoles were offset by 20 *m/z* to catch any product that had the characteristic loss of HF. Once a thorough list of degradation products was generated, chromatography was optimized and samples were quantitatively analyzed.

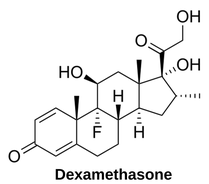
Samples (20 μL) were injected onto a Phenomenex Phenyl-Hexyl column [0.3 x 100 mm, 3.5 um] which was maintained at 40 °C. The column was eluted at 0.5 mL/min with a gradient of 10% acetonitrile in 20 mM ammonium formate, pH 3.8 (A) and 10% isopropanol in acetonitrile (B). Chromatographic separation was achieved with a linear gradient (time, % of solvent B): 0-9 min, 10.5-13.7% B; 9-17 min, 13.7-21% B; 17-22 min, 21-35% B; 22-23 min, 35-10.5% B; then isocratic for 10 minutes at 10.5% B to re-equilibrate the column.

The mass spectrometer was operated in the ESI$^+$ mode with the following settings: curtain gas, 50 psi; collision gas, high; ion spray voltage, 5500 V; source temperature, 600 °C; ion source gas 1 and 2, 30 psi; declustering potential, 40 V; and collision energy, 14 V. Dexamethasone and dexamethasone degradation products were quantified in the multiple reaction monitoring (MRM) mode using isotope dilution with d_4-dexamethasone as an internal standard. Dexamethasone and dexamethasone degradation product concentrations were determined by comparing the ratio of the HPLC-ESI$^+$-MS/MS peak area for each product to the d_4-dexamethasone peak area to the area ratios generated from a calibration curve.

Results

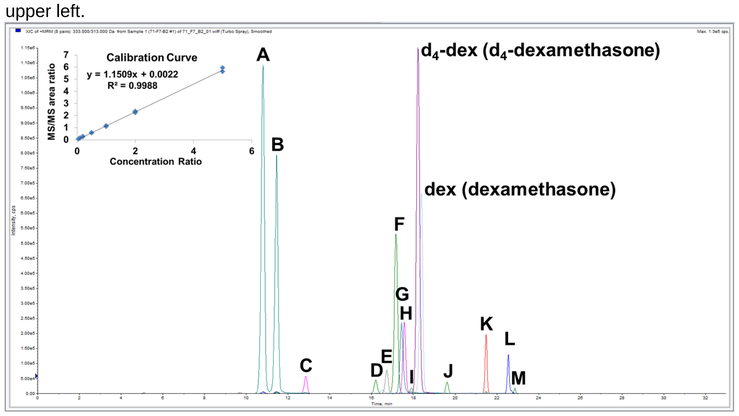
Table 1: Retention times and MS/MS transitions for dexamethasone and its degradation products.

$time_R$ (min)	Compound	MS transition
10.8	A	*m/z* 409 → *m/z* 389
11.6	B	*m/z* 409 → *m/z* 389
12.8	C	*m/z* 407 → *m/z* 387
16.2	D	*m/z* 363 → *m/z* 343
16.7	E	*m/z* 379 → *m/z* 359
17.2	F	*m/z* 363 → *m/z* 343
17.4	G	*m/z* 409 → *m/z* 389
17.6	H	*m/z* 407 → *m/z* 387
17.9	I	*m/z* 363 → *m/z* 343
18.2	d_4-dex (d_4-dexamethasone)	*m/z* 397 → *m/z* 377
18.4	dex (dexamethasone)	*m/z* 393 → *m/z* 373
19.6	J	*m/z* 363 → *m/z* 343
21.5	K (17b-carboxy-17a-formyloxy dexamethasone)	*m/z* 361 → *m/z* 341
22.6	L (17-oxo-dexamethasone)	*m/z* 333 → *m/z* 313
22.9	M	*m/z* 363 → *m/z* 343



Dexamethasone

Figure 1: Representative LC-MS/MS chromatogram for a release sample of a dexamethasone-PLGA implant incubated for 71 days; calibration curve embedded in the upper left.



Acknowledgment

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Figure 2: Dexamethasone degradation over time at 25, 37, and 45 °C (A); associated Arrhenius plot of the same data (B).

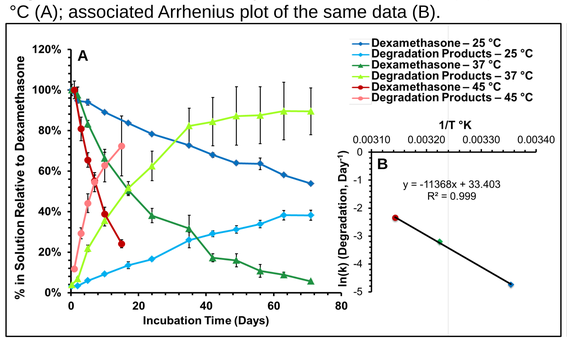


Figure 3: Distribution of dexamethasone and its degradation products as a function of incubation time.

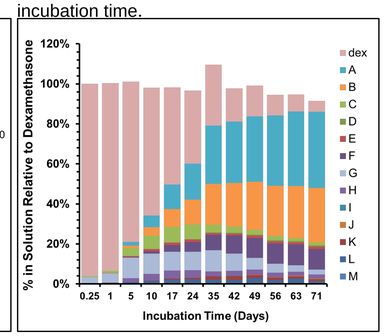


Figure 4: Dexamethasone release from dexamethasone-PLGA implants incubated at 37 and 45 °C (A); distribution of degradation products (B); simulated release using Boomer, generated from release, degradation, and medium replacement parameters (C).

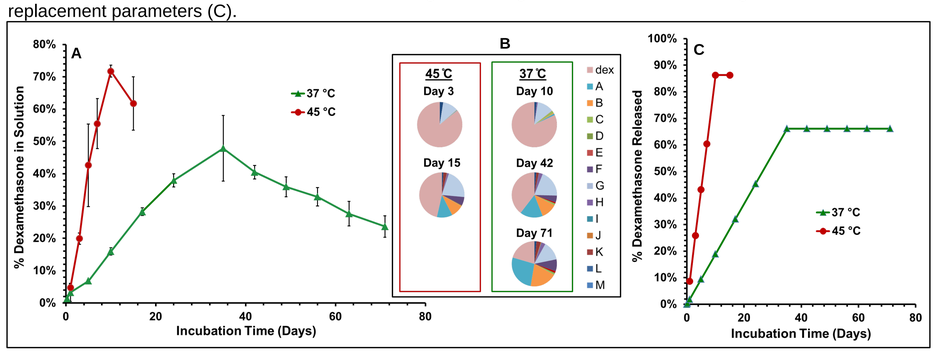
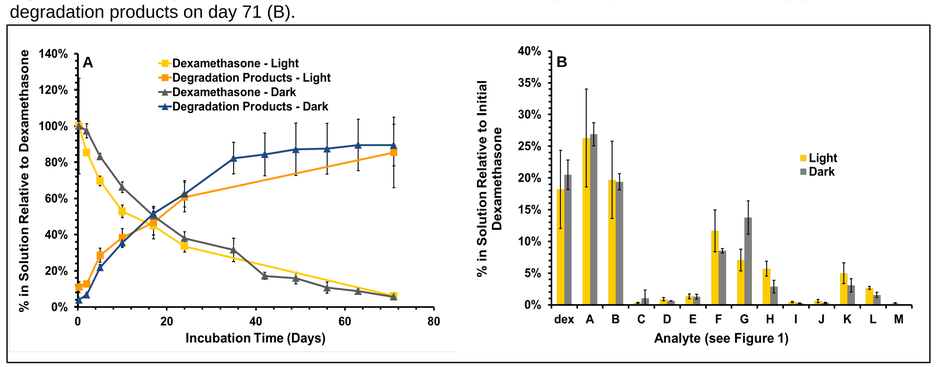


Figure 5: Effect of light on the degradation of dexamethasone during 71-day incubation at 37 °C (A); distribution of degradation products on day 71 (B).



Conclusions

- Dexamethasone degrades in PBS at pH 7.4, with the degradation rate increasing as a function of temperature.
- Dexamethasone degrades to a greater extent upon light exposure at early time points. These differences dissipate upon long term incubation up to 71 days. Light exposure does appear to change the distribution of the degradation products.
- The implant has a protective effect on dexamethasone. Only released dexamethasone appears to degrade.