Examination of steroid release from screw-in pacing leads

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Abstract

Although constant progress in pacing lead design and life time of pacemaker battery has contributed to the clinical success of this treatment option, the mechanism of drug release from steroid-eluting pacing leads is not completely understood. Besides, drug concentrations of steroids released into the heart-tissue remain unclear. Therefore, three different dissolution test methods were examined: a non-standardized (reagent tube), a compendial USP (paddle) and a hydrogel method. The experimental results indicate that dissolution from pacing leads is governed by a diffusion-controlled mechanism and occurs over a prolonged time. Furthermore, the integration of a hydrogel compartment seemed to have an impact on drug release leading to a decrease in release rate. Nevertheless, the experimental results emphasize different experimental parameters in dissolution testing may have a distinct influence on drug dissolution.

1 Introduction

Local drug delivery systems such as drug-eluting cardiac pacing leads or drug-eluting stents have become the most common surgical procedure in the management of pathologic cardiac arrhythmia or stenosis caused by coronary artery disease reducing morbidity and mortality of patients.1-2 Cardiac pacemakers and implantable cardioverter-defibrillators, both classified as medical devices, are cardiac electrical stimulation devices having one to three pacing leads using either active (screw-in) or passive (hook-in) anchorage. A monolithic drug reservoir, typically a silicone ring (collar), is mounted immediately behind the electrode of pacing lead. Pharmacological active substances such as glucocorticoids (dexamethasone acetate or dexamethasone sodium phosphate) are dispersed within the non-absorbable silicone matrix. Using this technique, the anti-inflammatory drug is delivered directly from the electrode/heart tissue-interface into the endomyocardium. Usually, lead implantation is associated with an acute and chronic foreign body response causing the development of a fibrous capsule around the electrode surface separating the electrode from excitable heart tissue.3,4 The inflammatory response depends on size, surface structure and material of the electrode and is related to a chronically increased stimulation threshold after 1 to 4 weeks postimplantation.5,6 Compared to steroid-free pacing leads, the implantation of drug-eluting devices lower the acute and chronic foreign body response leading to significantly reduced stimulation threshold.7,8

Despite the widespread clinical use of these well-established cardiac implants, the desired release into the heart tissue and distribution behaviour of the respective steroid remains unclear. Additionally, the mechanism of drug release from the silicone-matrix, which may be controlled by drug solubility (dexamethasone acetate: 15 µg/mL,9) or by the silicone matrix, is not completely understood. Commercially available standardized dissolution test methods as described in the Pharmacopoeia of the United States (USP) and in the European Pharmacopoeia (Ph. Eur.) or non-compendial methods such as the incubation in small tubes can be used to examine the in vitro dissolution and distribution processes.

In this work, the mechanism of drug release from steroid-eluting pacing leads was studied. Dissolution profiles of steroid-eluting collars and lead tip steroid-eluting collars investigated by USP apparatus II (paddle) and by non-standardized dissolution test were examined. Furthermore, using a hydrogel test setup biorelevant investigation of drug release and distribution behaviour becomes feasible. The model contains three compartments mimicking the situation in vivo: steroid-eluting pacing lead, the blood and the endomyocardium allegorized by a hydrogel compartment. In this study, experiments were performed to evaluate the necessity of a tissue-simulating dissolution test method.

2 Materials and methods

2.1. Chemicals

Micronized anhydrous dexamethasone acetate (DXA, content 100.6 %, Pfizer, USA) and dexamethasone (DX, content 98.9 %, Sigma-Aldrich Co., St. Louis, USA) were used. All chemicals were of analytical grade. Agarose was purchased from Sigma-Aldrich Co. (St. Louis, USA). Methanol of high pressure liquid chromatography (HPLC) grade was used to prepare the mobile phase and to dilute the samples. The mobile phase consisted of methanol (MeOH)/phosphate buffer pH 3.6 (PB). The pH of PB of 3.6 was adjusted with 10 % phosphoric acid after degassing.

2.2. Test collars

Steroid-eluting collars (SEC), lead tips with steroid-eluting collars (LTSEC) and steroid-eluting pacing leads (SEPL, figure 1) releasing DXA were supplied by BIOTRONIK SE & Co. KG (Berlin, Germany). LTSEC are test dummies for commercially available SEPL. The implants investigated in this study were identical in outer dimension and theoretical drug content (SEC: 1 mg; LTSEC: 1 mg and 750 µg).
2.3. Determination of drug content

The measurements were performed using a HPLC system with UV detection. The used HPLC system (Knauer, Berlin, Germany) consists of a solvent delivery module Knauer Smartline Pump 1000 (flow rate: 1 mL/min), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Smartline 2500 UV detector (λ: 242 nm), a Knauer Online Degasser, and a Knauer Smartline 3900 Autosampler. The used column was a Phenomenex Luna 3u C18 (3 µm particle size, 150 mm x 4.6 mm). The elution mixture was MeOH/water 65/35. Chromatograms and peak areas were recorded and integrated using Chromgate V 3.1.7 software. Drug concentrations were calculated as the sum of DXA and DX.

All spectrophotometrical quantifications of DXA were performed with a Cary 50 Scan (Agilent Technologies Inc., Santa Clara, California, USA) at 242 nm.

2.4. In vitro dissolution studies

Reagent tube release study. In vitro drug release measurements were performed in agitated reagent tubes filled with phosphate buffered saline (PBS) of pH 7.4 which is adapted to the blood pH. SEC (1 mg) were incubated with 5 mL PBS 7.4, protected from light to avoid DXA degradation by UV radiation and agitated in an incubator (300 rpm) at 37°C. At predetermined sampling points the SEC was transferred into freshly prepared dissolution media. After the last time point, the residual amount of DXA/DX of SEC was separately extracted by ultrasonification in tetrahydrofurane at room temperature. All samples were measured spectrophotometrically at 242 nm.

USP II study. Additionally, in vitro drug release of LTSEC (1 mg) were investigated (figure 2) using a compendial dissolution test setup USP II (paddle apparatus: PT-DT7, Pharma Test Apparatebau AG, Hainburg, Germany). To maintain DXA concentration below 10% of the saturation solubility (sink conditions), 900 mL dissolution media (PBS 7.4) at 37°C was used. Rotation speed was set to 50 rpm. SEC was tightly mounted on fiber glass wire to position the implants at the inner lateral surface of the dissolution vessel. For comparison, dissolution of 1 mg of DXA powder was investigated using the same test setup. Drug release was assayed by intermittently sampling the contents of dissolution media at various time intervals between 1 hour and 9 days. Replacement of sampled media (10 mL) occurred immediately after sampling. The amount of steroid concentration was measured directly via UV spectroscopy at 242 nm.

Hydrogel release studies. A hydrogel test method was used offering the opportunity to investigate DXA release from LTSEC (750 µg) into a 2% agarose hydrogel matrix (Ag-M). To prepare the hydrogel agarose powder was dissolved in PBS 7.4 at 70-80°C to form a 2% (w/w) agarose hydrogel having the interesting property of reverse thermal gelation. Afterwards, a layer of Ag-M solution was poured onto the bottom of a flask achieving sufficient hydrogel film thickness of 2 cm (r: 4 cm). LTSEC were placed in the hydrogel and the gel compartment was allowed to solidify. Finally, the Ag-M acceptor matrix was covered with 100 mL buffer solution (PBS 7.4). The test specimens prepared according to this method were kept for 1, 3, 7, 14 or 28 days, respectively, in an incubator at 37°C under gentle shaking (300 rpm). At predetermined time intervals aliquots of the supernatant were collected and stored at -20°C until HPLC analysis. Furthermore, at each end point the respective agarose matrices were extracted and the drug content of the gels as well as the residual content of the LTSEC was determined by HPLC. A schematic representation is depicted in figure 3.
2.2.4 Morphological characterization

To examine the physical states of SEC after release studies, a scanning electron microscope Phenom™ (FEI Company™, L.O.T.-Oriel GmbH & Co.KG, Darmstadt, Germany) was used. The samples of steroid/silicone-implants were dried in desiccators under vacuum for 24 h and coated with gold-palladium (mini sputter coater SC7620, Quorum Technologies Ltd., West Sussex, UK) in a sputtering atmosphere of argon.

3 Results

Reagent tube release study. The release profile of SEC showed a burst release with about 30% of the loaded DXA delivered during the first day possibly caused by drug particles located near the surface. After the initial burst, a decreased release rate can be observed. This reduction may be due to the drug particles embedded within the silicone matrix. These results agree with data reported previously by other authors. 11 Dissolution profile of cumulative DXA release from SEC is given in figure 5.

USP II study. An almost complete dissolution of reference powder was attained within 9 days (90% of theoretical amount of DXA/DX). In comparison, the release of steroid from LTSEC into PBS 7.4 reached 5% within the first day (20% after 9 days). Apparently, DXA release followed an outward diffusion-controlled mechanism and is not limited by its drug solubility. Cumulative drug release from LTSEC and dissolution profile of DXA powder is shown in figure 6.

Hydrogel release studies. After 1 day 0.4% (1.0%, 28 days) of overall detected drug amount was delivered into the hydrogel compartment, compared to concentration in media of 0.2% (3.7%, 28 days) and DXA/DX remained in the polymer-matrix 99.4% (95.3%, 28 days). The amount of DXA/DX released was plotted over time (figure 7).

In contrast to dissolution profiles obtained from reagent tube release studies (SEC), release from LTSEC was much slower. These differences may be caused by the investigated test specimens: Drug release from SEC occurs from all surfaces, whereas drug delivery from LTSEC occurs mainly from the outer surface. The inner and lateral surfaces are partially glued with the lead tip or end of electrode. High value of SD is related to the analytical procedure.

For the purpose of comparison, the dissolution testing of SEC in the absence of Ag-M was performed. Drug amount eluted from LTSEC (750 µg) in the absence of the hydrogel matrix into dissolution media was found to be 1.9% within the first day. Furthermore, after 28 d only 8% of...
overall detected amount of DXA/DX were observed. The results are shown in figure 8.

![Figure 8](image)

Figure 8 Amount of steroid in the compartment LTSEC and PBS 7.4 (mean ± SD, n=3 per data point).

Compared to experimental data obtained in the presence of Ag-M, release rate is slightly increased and totally delivered amount of DXA/DX into media raised from 3.7 % up to 8 %. Additionally, DXA/DX concentration (sum of DXA/DX detected in media and hydrogel, data not shown) is below those to be found only in dissolution media of the control experiment (figure 8, without the Ag-M compartment). These findings demonstrate that the inclusion of a hydrogel compartment influenced DXA release from SEC and LTSEC. The drug is transported within the hydrogel by diffusion due to DXA concentration gradient. Whereas steroid release without the hydrogel is additionally influenced by convection. Although, the mechanism of drug transport inside the extra- and intracellular matrix of heart tissue is dominated by diffusion. Therefore, a hydrogel compartment is likely better to mimic the endomyocardium. However, for dissolution testing of SEC and LTSEC releasing the desired drug slowly and over a prolonged time period an inclusion of a tissue-simulating compartment may have no important impact on drug release.

Surface morphology. SEM images of SEC are given in figure 9.

![Figure 9](image)

Figure 9 SEM images (cross-section) of SEC: Before dissolution testing (A, B), after drug extraction (C, D) and after reagent tube release studies (E, F).

Particles of undissolved drug substance distributed throughout the silicon matrix can be seen (A, close up on device surface: B). As expected, extraction of DXA resulted in the formation of pores near the surface (C, D). After dissolution testing (reagent tube release studies described above) smaller pores and fine cracks are observed which can be attributed to dissolution of DXA powder.

4 Conclusion
Results of release studies indicate that DXA release from SEC is governed by dissolution of the embedded drug particles and diffusion of dissolved steroid through media filled matrix pores as well as through the silicone-matrix. Consequently, DXA dissolution from collars is controlled by the device-matrix. In order to mimic in vivo conditions of drug release from these controlled drug delivery systems, dissolution tests should be performed with LTSEC or mounted SEC. The integration of a hydrogel compartment leads to no important differences in released drug amount. However, it enables dissolution testing of test collars under more realistic in vitro test conditions and is able to depict the process of drug diffusion.

5 Acknowledgement
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6 References