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Development of a vessel-simulating flow-through cell method for the in vitro evaluation of release and distribution from drug-eluting stents

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ABSTRACT

A novel in vitro dissolution test for drug-eluting stents (DES) based on the compendial flow-through cell was developed. The model contains an additional compartment simulating the vessel wall enabling the examination of drug release and distribution. The compartment consists of alginate hydrogel containing a central aperture forming the lumen for stent placement and media flow. The method was tested utilizing stents coated with hydrophilic (fluorescein sodium) and hydrophobic (triamterene) fluorescent model substances as well as the cytostatic drug doxorubicin hydrochloride and compared to standard dissolution methods. The results show the suitability of the developed method to observe drug release and distribution. The determination of model substance content in the compartments media, hydrogel and stent yielded differing distribution patterns for the model compounds and enabled the observation of redistribution processes. The dissolution profiles differed from the results of compendial dissolution testing. Besides lower endpoints and slower rises of media concentrations due to distribution into the hydrogel, the release rates from the stent coatings were altered. These findings emphasize the necessity to adapt dissolution testing for DES to the unique conditions influencing delivery to the vessel wall to learn more about local distribution and to anticipate the in vivo performance of DES.

1. Introduction

The application of drug-eluting stents (DES) in the treatment of coronary artery disease has tremendously reduced the rate of in-stent restenosis compared to the conventional bare-metal stent (BMS) [1,2]. The two drug-device combinations that have been approved by the FDA up to date contain the extremely hydrophobic drug substances paclitaxel (log *P* 4.4 [3], solubility in water 1 μg/ml [4]) and rapamycin (log *P* 5.8 [5], solubility in water 2.6 μg/ml [6]), respectively. More hydrophilic candidate drugs such as dexamethasone (log *P* 1.9 [7], solubility in water 100 μg/ml [8]) on the other hand have not shown the desired clinical outcomes in spite of promising results in cell culture and animal models [9,10]. The inferiority of compounds with higher hydrophilicity might be related to higher diffusive washout [11]. Furthermore, paclitaxel and rapamycin specifically bind to intracellular proteins which also seems to be a crucial efficacy affecting criterion [12]. Besides these factors governing distribution processes between blood and the vessel wall, the distribution within the arterial tissue is of particular interest. Findings of Hwang et al. [11] suggest that drug release from stents may lead to large concentration gradients in the vessel wall in dependency of the strut position, possibly ranging from toxic to ineffective. Accordingly, local drug release and resulting tissue distribution need to be examined thoroughly. The examination of in vivo release and distribution is, however, limited by the fact that drug concentrations in humans cannot reliably be estimated due to low concentrations and the fact that blood levels do not necessarily correspond to drug concentration at the target organ [13]. For these reasons in vivo determination of drug release is often only feasible through stent removal and analysis of the residual content or analysis of tissue specimens in animal models. Discrepancies between data obtained from animal models and clinical outcome in humans have been reported, though [14,15]. Due to these limitations to in vivo examination of release and distribution profiles, a suitable in vitro model would be extremely valuable for the preclinical evaluation of DES. Of the compendial methods for dissolution testing the reciprocating cylinder and the flow-through cell have been considered for controlled release parenterals [16,17]. Furthermore, noncompendial methods have been used to evaluate drug release from DES [18–22]. These methods commonly comprise the immersion of the DES in small volumes of liquid (1–15 ml) at 37 °C with or without agitation. The liquids employed as dissolution

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media range from phosphate buffered saline or isotonic sodium chloride solution to porcine blood plasma and may contain additives such as surfactants or cosolvents. All of these methods allow for the examination of release into an aqueous compartment but lack an additional compartment which simulates the vessel wall and its possible influence on drug release. Therefore, it was the aim of this work to develop a dissolution test method which takes the unique conditions influencing local drug delivery to the vessel wall, e.g. the placement of the stent in direct contact with the vessel, into account.

The developed dissolution test was evaluated using model stents that were dip-coated with two different fluorescent model substances. Fluorescein sodium, a commonly in microscopy and dye tracing employed fluorophore (λ_{ex} 490 nm/λ_{em} 515 nm [23]), was used as a model hydrophilic substance (log *P* −1.52 [24], freely soluble in water). Triamterene, a potassium-sparing diuretic, was chosen as a model compound due to its hydrophobicity (log *P* 1.25 [25], solubility in water 28 µg/ml [26]) and its intrinsic fluorescence (λ_{ex} 370 nm/λ_{em} 434 nm [27]). A blend of water-insoluble but diffusible polymethacrylates (ammonio methacrylate copolymer type A and B) was used as the model coating polymer for these substances. Furthermore, stents spray-coated with the cytostatic drug doxorubicin hydrochloride (log *P* 0.71 [28], solubility in buffer solution pH 7.5 200 µg/ml [29]) embedded in poly-L-lactide, a polymer widely used for implants including stents [21,30,31], were investigated with the developed test system. The topoisomerase II inhibitor doxorubicin exhibits intrinsic fluorescence with an excitation maximum at 479 nm and emission maxima at 560 nm and 593 nm [32].

2. Materials and methods

2.1. Materials

Sodium alginate according to Ph. Eur. specification was obtained from Fagron GmbH & Co. KG (Barsbüttel, Germany). BMS as well as balloon catheters were generously provided by Cortronik GmbH (Rostock-Warnemünde, Germany). Eudragit® RL and RS were a gift from Roehm GmbH (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Modification of the compendial flow-through cell

The standard flow-though cell for tablets (diameter 22.6 mm) was adapted as shown in Fig. 1. The conical part was shortened 10 mm thus creating a shoulder onto which an acrylic glass disc (diameter 22.5 mm, height 4.5 mm) was placed. The disc was equipped with a sealing ring to prevent leakage at the sides and a tapped through hole in the centre. A stainless steal rod was screwed into the thread and served as a placeholder during gel formation to create an opening in the gel simulating the artery lumen.

2.3. Stent coating

To obtain fluorescein sodium or triamterene coated stents, stainless steel stents were mounted on a mandrel and dipped into the polymer solution containing the dissolved or suspended fluorescent model drug. Fluorescent probe concentration ranged between 20% and 25% of total solid content. Coating layer mass was approximately 200 µg as determined by weighing. The polymer solution consisted of a blend of ammonio methacrylate copolymer type A and B (Eudragit® RL/Eudragit® RS, ratio 3:7) dissolved in isopropyl alcohol 75% (m/m). Doxorubicin hydrochloride was dissolved in dimethyl sulfoxide, blended with a solution of poly-L-lactide in chloroform and spray-coated onto stainless steel stents. The coating layer mass averaged 500 µg and the doxorubicin hydrochloride content in the coating amounted to 30% of the total solid content. Coatings were inspected in the fluorescence microscope (Axiovert 200, Carl Zeiss MicroImaging GmbH, Jena, Germany, triamterene: λ_{ex} 365 nm/λ_{em} ≥ 397 nm, fluorescein sodium: λ_{ex} 450–490 nm/λ_{em} 515–565 nm, doxorubicin hydrochloride: λ_{ex} 546 nm/λ_{em} ≥ 590 nm) and microscopic images (Fig. 2) were obtained using AxioCam HrC (Carl Zeiss MicroImaging GmbH, Jena, Germany).

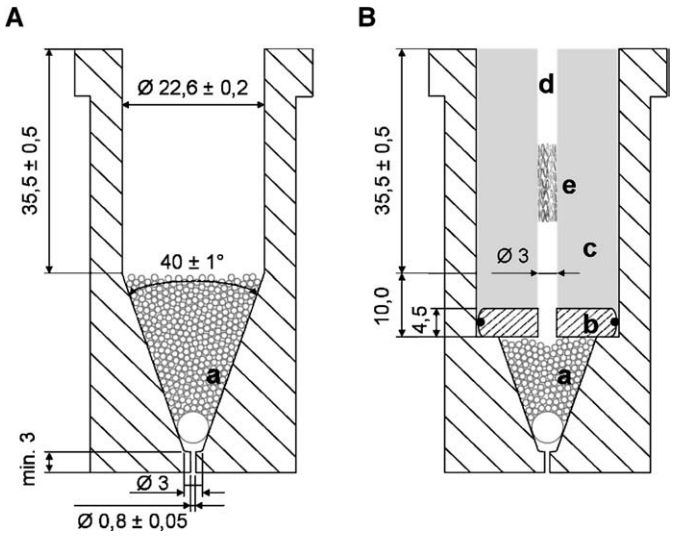

Fig. 1. Scheme of the compendial flow-through cell according to Ph. Eur. (A) and the vessel-simulating flow-through cell (B), measures in mm, a glass beads, b acrylic glass disc, c hydrogel, d aperture for media flow, e stent.

2.4. Hydrogel preparation and dissolution testing

Calcium alginate hydrogel was formed by an internal gelation method based on the slow release of free calcium ions from a poorly soluble salt [33]. A sequestrant was used to delay gel formation until homogeneous distribution of the salt within the sol had been achieved and the mixture had been poured into the mold. The gels were prepared by suspending 0.15 g calcium sulfate dihydrate in 2.0 g of sodium triphosphate solution 2.5% (m/m) in purified water. This suspension was added to 16.5 g of a solution of sodium alginate in purified water 3% (m/m). This resulted in a water content of the gel of approximately 96%. After thorough blending the mixture was poured into the prepared flow-through cell and allowed to set for 15 min. After removal of the placeholder rod a stent was inserted into the formed aperture in the hydrogel via balloon catheter and dilated (6 atm, 15 s). Dissolution testing was conducted in a closed system setup at 37 °C. 250 ml dissolution media phosphate buffered saline pH 7.4 (PBS) according to Ph. Eur. were circulated by means of a peristaltic pump at a flow rate of 35 ml/min. In the case of doxorubicin hydrochloride coated stents a media volume of 100 ml was employed due to analytical limitations.

Samples were withdrawn at predetermined times. 200 µl were transferred to a 96 well plate and fluorescence intensity was measured using a fluorescence reader (Fluroskan II, Labsystems, Helsinki, Finland, fluorescein sodium: λ_{ex} 485 nm/λ_{em} 538 nm, triamterene: λ_{ex} 355 nm/λ_{em} 460 nm, doxorubicin hydrochloride: λ_{ex} 485 nm/λ_{em} 590 nm). Calibration was performed with every experiment. All measurements were performed in duplicate. Reduction of media volume due to sampling and evaporation was taken into account in the calculation of compound release. After the intended incubation period the stent was removed from the hydrogel, transferred to fresh PBS and incubated for at least 48 h. The media was removed after 24 h and replenished with fresh PBS. Samples of the incubation buffer were analyzed to determine the residual content of the fluorescent probe in the stent coating as described above. In the case of doxorubicin hydrochloride coated stents the residual amounts were determined after elution with dimethyl sulfoxide instead of PBS. In order to estimate the amount released into