European Journal of Pharmaceutical Sciences 93 (2016) 351–359

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Evaluation of ion exchange processes in drug-eluting embolization beads by use of an improved flow-through elution method

Tanya Swaine [a], Yiqing Tang [b], Pedro Garcia [b], Jasmine John [b], Laura J. Waters [a], Andrew L. Lewis [b,*]

[a] Department of Pharmacy, School of Applied Science, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UK
[b] Biocompatibles UK Ltd, Lakeview, Riverside Way, Watchmoor Park, Camberley GU15 3YL, UK

ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form 9 August 2016
Accepted 9 August 2016
Available online 12 August 2016

Keywords:
Drug-eluting beads
Embolization
Flow-through elution system
Doxorubicin
Ion-exchange

ABSTRACT

An improved method for evaluating drug release behaviour of drug-eluting embolization beads (DEBs) was developed utilizing an open-loop flow-through system, in which the beads were packed into an occlusive mass within the system and extracted with a flowing elution medium over time. Glass beads were introduced into the beads mass in order to ensure laminar flow, reduce dead volume and improve reproducibility by compensating for swelling phenomena. The effects of glass bead ratio, elution medium flow rate and ion concentration, DEB size and drug concentration and drug type (doxorubicin and irinotecan) were evaluated using DEB composed of a sulfonate-modified polyvinyl alcohol hydrogel (DC Bead™) as the test article. The rate and amount of drug elution from the packed beads was affected by flow rate, the bead size and initial loading dose. The raw data from the concentration profile analysis provided valuable information to reveal the drug elution behaviour akin to the pharmacokinetic data observed for embolized beads (yielding *in vitro* C_{max} and t_{max} data) which was complementary to the normal cumulative data obtained. A good correlation with historical reported *in vivo* data validated the usefulness of the method for predicting *in vivo* drug elution behaviour.

1. Introduction

One of the most important characteristics of a novel drug-eluting system is the kinetics of the release of the active pharmaceutical ingredient (API) from the matrix or reservoir in which it is contained. A consistent, reliable and robust method carried out under controlled laboratory conditions is critical for both the development and quality control of such products. It will allow for comparative performance assessments across other similar formulations for product optimization, bench-marking purposes or bioequivalence arguments, for instance, in addition to providing key *in vitro* release testing (IVRT) data that are required for release of product for sale (Brown et al., 2011). Such testing involves subjecting the drug-eluting system to carefully controlled conditions that will facilitate drug release, with subsequent monitoring and measurement of the elution over time to obtain kinetics of the release profile and often a total amount released.

When modelling how the system may perform in the body, it is necessary to attempt to select the *in vitro* conditions that best simulate the *in vivo* environment in which the system will be located. This will vary considerably depending upon the route of administration and will need very different conditions when evaluating topical, oral, parenteral and implantable release systems for example. When being used for quality control purposes, the method can be used to assess conformance of the system to a product specification and the stability of API release over time (Anand et al., 2011). In this case therefore, it is essential that the test conditions are selected in order to be capable of demonstrating any deviations of API elution profile from that expected for a product conforming to specification, which could be a consequence and indicative of a change in the drug-eluting system. The method is therefore an essential test for the evaluation of a system that releases API and is known as dissolution testing for oral formulations, but in recent years is also referred to as IVRT or drug elution testing for the less conventional drug delivery systems, such as drug-device combination products (Sree Lakshmi and Badarinath, 2013).

Drug-eluting embolization beads have been in clinical use for over a decade now (Lewis et al., 2006), with several types commercially available for the treatment of hypervascular tumors by occlusion of the blood supply coupled with a sustained controlled locoregional drug release (Liapi et al., 2007; Malagari, 2008; Nicolini et al., 2011). These products are made from a variety of different polymer microspheres with the ability to load and release certain drugs, usually by an ion-exchange mechanism (Anand et al., 2001; Lewis et al., 2007). The different chemical composition of the matrices means that these products have different drug loading capacities and elution kinetics which therefore translate into differences in drug pharmacokinetics and bioavailability parameters and hence may impact on their clinical performance. It is therefore becoming ever-more important to have drug elution methods

* Corresponding author.
E-mail address: andrew.lewis@btgplc.com (A.L. Lewis).

http://dx.doi.org/10.1016/j.ejps.2016.08.020

that will allow direct comparison between products, in addition to better predicting the *in vivo* performance of these systems. This is not straightforward however, as physicochemical differences in the properties of the products, such as size, morphology and density can impact on their behaviour during analysis making direct comparison in performance problematic.

The IVRT of implantable drug-device combination products can be evaluated by immersing the system, usually with stirring, in a suitable elution medium (sometimes modified with additives to help in the solubilization of particularly low-solubility drugs). The medium may be exchanged periodically to prevent saturation with drug, and is sampled periodically for analysis determination of the elution kinetics. This type of experiment can be performed with USP type II apparatus or similar, which is well-established in regulatory guidelines and the US Pharmacopeia (Chapter <711>). As an alternative to exchange of the elution medium, it may instead be circulated around or through the system depending upon its configuration (*i.e.* using a flow-through apparatus, such as a USP type IV apparatus) (Andhariya and Burgess, 2016). Drug-eluting beads have been evaluated using both of these methods; the former being more useful for quality control purposes but inappropriate for *in vivo* correlation (Gonzalez et al., 2008), whereas the latter was unable to demonstrate complete drug elution from the systems under study due to experimental limitations (Jordan et al., 2010). A T-apparatus was proposed as an alternative method that better emulates the embolization environment by provision of diffusion and convection zones representing drug diffusion from the beads through the vessel wall and surrounding tissues and then its removal in blood flowing through more distant patent vessels (Amyot et al., 2002). This method has proved useful in predicting the first 24 h of drug release into the systemic circulation, producing level A *in vitro: in vivo* correlations (IVIVC) (Gonzalez et al., 2008). The method is, however, cumbersome and not without technical limitations. Although flow-through methods have been used in the study of doxorubicin elution from ion exchange microspheres (Willmott et al., 1985; Cremers et al., 1990), detailed studies based on the factors such as flow rate, drug type and loading dose *etc.* on the mechanistic evaluation of DEB characteristics are still lacking. Moreover, there is no study of the correlation between the *in vitro* elution modelled using flow-through methods and *in vivo* drug release data. Herein we report on the development of an improved elution method based on open loop flow-through mechanism for evaluation of DEBs which overcomes some of the short-comings of previously-reported methods and will allow a better comparison between the performances of different products despite their differing characteristics.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride (Dox, Hisun, China) and irinotecan hydrochloride (Iri, ScinoPharm, China Taiwan) were obtained as pure powders (>99% purity) and dissolved in deionised water at the desired concentration to obtain stock solutions for bead loading experiments. Hydrogel beads used in the elution were DC Bead™ (Biocompatibles UK Ltd., a BTG International group company, Farnham, UK). Glass beads with size range 150–220 μm used in the experiment were purchased from Sigma. Phosphate buffered serology saline was supplied by Source BioScience (UK) and degassed using helium (BOC, UK) prior to use.

2.2. Drug loading method

Samples of the beads under study (DC Bead™: 70–150 μm, 100–300 μm, 300–500 μm and 500–700 μm size ranges) were loaded with a target dose of the desired drug. The bead samples were first transferred from their product vials to a 10 mL measuring cylinder. 1 mL of beads was measured into a 10 mL glass vial. After as much residual packing solution was removed as possible from the beads by using a pipette with a cotton filter on its tip, drug loading was then initiated by the addition of the drug solution at the desired concentration. The actual amount to be added was calculated based on the measured concentration of the drug loading solutions and pipetted accurately into the vial. The vial was gently agitated several times during loading and complete loading (>99%) was confirmed by UV–Visible spectrophotometric analysis of the loading solution.

2.3. Drug elution method development and set-up

Drug-loaded bead samples prepared in Section 2.2 were evaluated using an open loop flow-through system as depicted in Fig. 1. This system consists of PBS stock, a peristaltic pump (ISMATEC, Germany) with silicon tubing (ID 0.094″, OD 0.156″, Cole-Parmer, USA), a flow-through elution cell (ID 14 mm, OD 20 mm, length 125 mm), a water bath with temperature control unit (HAAKE SC100, Thermo Scientific, USA), a Varian Cary® 50 UV–Vis spectrophotometer (Agilent Technologies, Australia), and waste collection. The glass beads (150–212 μm, Sigma, USA) and drug loaded beads were mixed uniformly and sandwiched between two filter membranes (pore size 27 μm, SeFar Medifab, UK) in the elution cell. As discussed in Section 3, the introduction of glass beads is in order to create some interstitial space between beads with the aim of separating and suspending beads as well as allowing for any size changes during elution to be accommodated. In the case without glass beads, the drug-loaded beads were directly placed between the filter membranes with a 6–7 mm gap allowing beads swelling. Deionised water was used here as packing medium to avoid pre drug leaching, and during bead packing, air bubbles were carefully avoided from being introduced into the system. Further measures were adapted by using helium-sparged PBS and one more layer filter to stop any air bubble entering the elution cell.

After assembling the system, the PBS was pumped through the elution cell at 37 °C by using selected speeds between 0.5 and 45 mL/min. The eluted drug was passed through UV spectrophotometer, and monitored at 483 nm for Dox and 369 nm for Iri, respectively. Due to the high extinction coefficient of Iri and its relatively high initial rate of elution, eluted irinotecan was passed through a quartz cuvette (Hellma Analytics, Germany) with 0.50 mm path length (Fig. 1) for concentration measurement.

2.4. Doxorubicin elution through a packed bead column along the direction of flow

The study of doxorubicin distribution during flow-through elution was carried out by packing 0.6 mL of beads containing 37.5 mg/mL doxorubicin into three silicon tubes with a diameter of 3 mm and length of 150 mm, respectively. The initial packed length of drug loaded beads was 4.4 cm. The end of the silicon tubing was installed with a 10 μm filter to hold the beads during the elution. The bead packed tubing was immersed into 37 °C water bath and saline was driven through it using a peristaltic pump. At different predetermined time points, the tubes were sequentially sectioned into four equal parts according to the total length of the packed section using a sharp blade. The sectioned beads were transferred into a pipette column and extracted by DMSO-NaCl solution (NaCl concentration 1%). The extracting solution was diluted with deionised water and measured by UV spectrophotometry at 483 nm. The data were compared to a standard curve obtained under the same conditions to obtain the drug concentration in beads within each section.

The data collected were processed using Excel. Firstly the absorbance data were converted to concentration by comparing to the standard solution. Then the area under curve (AUC) and cumulative amount of drug eluted were calculated against time in minutes. The average elution curve was drawn by taking the mean of three replicate