Preparation and characterisation of vandetanib-eluting radiopaque beads for locoregional treatment of hepatic malignancies

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A B S T R A C T

Since their introduction around a decade ago, embolic drug-eluting beads (DEBs) have become a well-established treatment option for the locoregional transarterial treatment of hepatic malignancies. Despite this success, the therapy is seen to be limited by the choice of drug and more effective options are therefore being sought. These include the small molecule multi-tyrosine kinase inhibitors (MTKi), which exert an anti-angiogenic and anti-proliferative effect that could be highly beneficial in combating some of the unwanted downstream consequences of embolization. Vandetanib is an MTKi which acts against such targets as vascular endothelial growth factor receptor (VEGFR) and epithelial growth factor receptor (EGFR) and has demonstrated modest activity against hepatocellular carcinoma (HCC), albeit with some dose-limiting cardiac toxicity. This makes this compound an interesting candidate for DEB-based locoregional delivery. In this study we describe the preparation and characterisation of vandetanib DEBs made from DC Bead™ and its radiopaque counterpart, DC Bead LUMI™. Drug loading was shown to be dependent upon the pH of the drug loading solution, as vandetanib has multiple sites for protonation, with the bead platform also having a fundamental influence due to differences in binding capacities and bead shrinkage effects. Fourier transform infrared (FTIR) spectroscopy and energy dispersive X-ray (EDX) Spectroscopy confirmed drug interaction is by ionic interaction, and in the case of the radiopaque DEB, the drug is distributed uniformly inside the bead and contributes slightly to the overall radiopacity by virtue of a bromine atom on the vandetanib structure. Drug release from both bead platforms is controlled and sustained, with a slightly slower rate of release from the radiopaque bead due to its more hydrophobic nature. Vandetanib DEBs therefore have suitable characteristics for intra-arterial delivery and site-specific sustained release of drug into liver tumours.

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1. Introduction

The current standard of care for intermediate hepatocellular carcinoma (HCC) is transarterial chemoembolization (TACE), an image guided procedure in which a chemotherapeutic agent is injected via the hepatic artery into the tumour feeding blood vessels. This is followed by an embolic material which occludes the vessels, with the aim of starving the tumour of oxygen and nutrients (Bruix et al., 2004). TACE may also be performed using drug eluting beads (DEB): embolic microspheres capable of loading positively charged drugs and releasing them local to the tumour site via ion exchange, thus reducing systemic drug exposure (Lewis and Holden, 2011).

The drug of choice for loading into DEB is usually doxorubicin, a cytotoxic agent which intercalates with DNA, interfering with replication (Gewirtz, 1999). However, it has been shown that hypoxic conditions can lead to doxorubicin resistance in HCC cells (Cox and Weinman, 2015). Moreover, tumour hypoxia induced by embolization therapy leads to upregulation of pro-angiogenic pathways contributing to the formation of new blood vessels (Liang et al., 2010; Rhee et al., 2007). Anti-angiogenic strategies are therefore being considered in combination with DEBs to improve treatment outcomes.

Several trials combining TACE with oral anti-angiogenic therapy such as sorafenib, sunitinib or bevacizumab are underway or completed with some promising results, however there remain toxicity issues associated with oral use of these agents (Lencioni et al., 2016; Erhardt et al., 2014). DEBs provide an opportunity for local delivery of these agents, allowing for high local doses and reduced toxicity compared with oral treatment. Forster et al. were the first to demonstrate that DEBs could be loaded with a combination of drugs (Forster et al.,...
2012), not only to provide the benefit of local delivery of a cytotoxic drug such as doxorubicin, but also concomitant delivery of an agent with a complimentary mode of action such as rapamycin in order to downregulate expression of Hypoxia Inducible Factor Alpha (HIF-1α) and consequently induction of angiogenesis. Sakr et al. have more recently described a layer-by-layer process for loading bevacizumab onto DC Bead™ (Sakr et al., 2016) for controlled locoregional delivery of a monoclonal antibody that inhibits Vascular Endothelial Growth Factor-A (VEGF-A), a chemical signal for angiogenesis. It is however, the small molecule multi-tyrosine kinase inhibitors that have attracted most interest, given the approval of sorafenib (Nexavar®, Bayer-Onyx) for the treatment of advanced HCC. Sorafenib drug-eluting peptide nanocomposites (Park et al., 2016) and poly(lactide-co-glycolide) microspheres (Chen et al., 2014; Chen et al., 2015), both with magnetic resonance imaging (MRI) capability have been described for intra-arterial delivery to treat HCC. Poly(D,L-lactic acid) microspheres have also been prepared and loaded with either sorafenib or together with cisplatin and showed that the drug combination had a faster release rate (Wang et al., 2015) and was more efficacious in cell assays (Wang et al., 2016) compared to either drug alone. Lahti et al. described the loading of LC Bead with both sorafenib (Lahti et al., 2015a) and sunitinib (Lahti et al., 2015b) but both processes were inefficient. Fuchs et al. have reported on a much more comprehensive appraisal of sunitinib loading into DC Bead™ (Fuchs et al., 2015), correlation of in vitro release with in vivo pharmacokinetics (Fuchs et al., 2014) and evaluation of antitumor effects in a rabbit VX2 embolization model (Bize et al., 2016). Whilst these studies show great promise and demonstrate feasibility for the locoregional delivery of MTKis from DEBs, concerns remain over the severe toxicity of sunitinib in the liver (Cheng et al., 2013) and alternative suitable MTKi candidates have been sought.

Table 1 outlines a short selection from a broad list of options that have been considered for MTKi loading into DEBs, taking into account drug structure, properties and mode of action. Herein we describe a feasibility study undertaken with one of these candidates, vandetanib. Vandetanib is a multi-tyrosine kinase inhibitor (MTKi) which selectively inhibits vascular endothelial growth factor receptor 2 (VEGFR-2), epidermal growth factor receptor (EGFR), and rearranged during transfection (RET) tyrosine kinase (Morabito et al., 2009). Common adverse events associated with the oral delivery of vandetanib include diarrhea and rash, which can occasionally be severe. Prolongation of the QT interval (a measure of the time between the start of the Q wave and the end of the T wave in the heart’s electrical cycle and a marker for the potential of ventricular tachyarrhythmias) has also been observed (Tsang et al., 2016). The VEGF and EGFR pathways have both been implicated in the pathogenesis of HCC, and a phase II trial of vandetanib in advanced unresectable HCC patients showed a suggestion of improvement in progression free survival and overall survival (Hsu et al., 2012). There is therefore, a strong rationale for the locoregional delivery of this drug.

In this study the feasibility of loading vandetanib into DEB was investigated using both DC Bead™ and the recently approved radiopaque DEB DC Bead LUMÍ™ (Biocompatibles UK Ltd., Farnham, UK). This has resulted in an understanding of the interactions between vandetanib and the hydrogel network of the microspheres and the physicochemical characteristics of the resulting vandetanib-eluting beads in terms of maximum drug loading capacity, morphology, drug distribution and release.

2. Materials and methods

2.1. Materials

DC Bead™ and DC Bead LUMÍ™, both of diameter range 70–150 μm, were provided by Biocompatibles Ltd. (Farnham, UK). Vandetanib base was provided by Astra Zeneca (Macclesfield, UK, >99% purity).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mode of action</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axitinib</td>
<td>Potent VEGF inhibitor, inhibits breast cancer growth, prevents neangiogenesis in 9 L tumours, activity in refractory met renal cell carcinoma and HCC</td>
<td>DMSO + DMF soluble Solubility of over 0.2 μg/mL in AQ media with a pH 1.1–7.8, pKa 4.8</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>Active inhibitor of Bcr-Abi, inhibits tumour cell growth, angiogenesis, growth factor expression</td>
<td>DMSO + EIOH soluble, poorly water soluble</td>
</tr>
<tr>
<td>Canertinib dHCl</td>
<td>Potent pan-erb B tyrosine kinase inhibitor, irreversible TK inhibitor, radiosensitizer</td>
<td>10 mg/mL water solubility, DMSO ≥5.6 mg/mL, logP 3.05</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>SRC and BCR/ABL tyrosine kinase inhibitor, Lyn &amp; Src kinase inhibitor</td>
<td>Very poorly water soluble (0.0128 mg/mL), pKas 3.1, 6.8, logP 1.8</td>
</tr>
<tr>
<td>Dovitinib</td>
<td>MTKi of FGR 3, inhibits tumour growth in xenograft models of colon cancer</td>
<td>DMSO soluble, poorly EIOH soluble</td>
</tr>
<tr>
<td>Erlotinib HCl</td>
<td>Targets EGFR, potent inhibitor of AK2-V617F, ”Tarceva” approved for NSCLC, pancreatic and others</td>
<td>Slight water and methanol solubility, pKa 5.42, logP 2.7</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>EGFR TK inhibitor. ”Iressa” approved for NSCLC</td>
<td>Solubility 0.027 mg/mL, sparingly water soluble at pH 3, pKas 5.4, 7.2, logP 3.2</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Inhibits particular TK enzymes, used to decrease bcr-abl TK activity.</td>
<td>DMSO and water soluble, poorly EIOH soluble, logP 3</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>EGFR and HER2/neu dual TK inhibitor, potent EGFR kinase inhibitor, can restore tamoxifen sensitivity.</td>
<td>Poorly water soluble (0.0223 mg/mL), DMSO soluble, logP 5.4</td>
</tr>
<tr>
<td>Lestaurtinib</td>
<td>Potent inhibitor of several TKs FLT-3, TrkA, clinical activity in AML pts. with FLT-3 mutations</td>
<td>Very poorly water soluble</td>
</tr>
<tr>
<td>Masitinib</td>
<td>Protein TK inhibitor. phase III in dogs with canine mast cell tumours</td>
<td>Soluble in DMSO ≥95 mg/mL, water ≥1.2 mg/L</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>Selective BCR-ABL inhibitor and of proliferation of haematopoietic cells in CML and ALL TK inhibitor Ph I in CML. ”Tasigna™”</td>
<td>Poorly water (0.00201 mg/mL) and poorly EIOH soluble, DMSO soluble, logP 4.51</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Selective multitargeted TK inhibitor, targets VEGFR, PDGFR and c-kit.</td>
<td>Limited solubility in warm DMSO, poorly water soluble (0.0433 mg/mL) logP 3.59</td>
</tr>
<tr>
<td>Sunitinib Malate</td>
<td>Selective inhibitors of MTKs, inhibits FLT3-ITD pathway and angiogenesis by targeting VEGFR-2 and PDGFR-B. ”Nexavar®” approved renal cell carcinoma and HCC</td>
<td>Water soluble at pH 1.2–6.8, fluorescent, weak base pKa 8.95, logP 5.2</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>VEGFR-dependent tumour angiogenesis inhibitor, EGFR &amp; RET-dependent proliferation inhibitor. ”Zactima®” for follicular, medullary, anaplastic, adv/met papillary thyroid cancer</td>
<td>DMSO soluble, poorly water soluble (0.008 mg/mL), soluble at lower pHs, logP 5.01</td>
</tr>
<tr>
<td>Vatalanib DiHCl</td>
<td>Selective inhibitor for</td>
<td>Water soluble (10 mg/mL),</td>
</tr>
</tbody>
</table>
Table 1 (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mode of action</th>
<th>Properties*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFR-1&amp;2 TK + PDGFb-c, Kit &amp; c-FMS</td>
<td>DMSO soluble (85 mg/mL)</td>
<td></td>
</tr>
</tbody>
</table>

* Information sourced from www.drugbank.ca or individual drug FDA labels.

2.2. Loading of beads with vandetanib solution

As vandetanib exhibits pH dependent solubility, solutions were prepared by first dissolving the powdered drug in 0.1 M hydrochloric acid to 70% of the required volume. The solution was then adjusted to the required pH by dropwise addition of sodium hydroxide, before making up to volume with purified water. By this protocol, solutions at pH 2.1 and 4.4 were prepared at a concentration of 20 mg/mL. Solutions at pH 6.8 were prepared at a concentration of 5 mg/mL due to limits in solubility.

To load beads with vandetanib, the packing solution was removed from bead vials and vandetanib solution was added. Incubation with the loading solution was performed at room temperature under agitation for a minimum of 2 h. Depleted loading solution was removed and the beads washed 3 times in deionised water. To determine loading efficiency, vandetanib concentration in the depleted loading solution and washings was quantified by HPLC, using a Hypersil Gold C18 column (Thermo Scientific, UK) and PDA detector at a wavelength of 254 nm. Mobile phase was a gradient of 0.1% TFA v/v in methanol; 0.1% TFA v/v in purified water and 0.1% TFA v/v in methanol; flow rate 1 mL/min.

2.3. Bead morphometry

Bead samples before and after vandetanib loading were examined using an Olympus BX50 optical microscope with attached Colorview III camera. The diameter of 200 beads per sample was recorded using Stream software (Olympus, Southend on Sea, UK). Bead density was determined using a displacement assay described in detail previously (Duran et al., 2016).

2.4. Elemental mapping using SEM-EDX

DC Bead LUMI™ was loaded with the maximum vandetanib dose of 135 mg/mL of sedimented beads. Bead samples were embedded in Tissue-Tek optimum cutting temperature compound (Sakura, Thatcham, UK) on liquid nitrogen and transferred to −80 °C to ensure thorough freezing. Samples were sectioned in a cryostat (Leica, Milton Keynes, UK) in 10 μm thick sections and mounted on SEM stubs with carbon pads. Samples were coated with 4 nm platinum using a Q150T turbo molecular-pumped sputter coating system (Quorum, Lewes, UK) prior to SEM-EDX analysis. SEM imaging was carried out on a Zeiss SIGMA FEG-SEM (Zeiss, Cambridge, UK) scanning electron microscope equipped with an Oxford Instruments 80 mm² X-Max energy dispersive X-ray analysis system. The sample was left in the SEM for several minutes prior to imaging to ensure the small amount of water present in the section was removed under the high vacuum. Elemental mapping and spectra were acquired for iodine, bromine, fluorine and sulfur on unloaded DC Bead LUMI™ and DC Bead LUMI™ loaded with vandetanib at maximum capacity.

2.5. Vandetanib-bead interactions using Fourier transform infra-red microscopy

DC Bead LUMI™ loaded with different doses of vandetanib were embedded and sectioned into 10 μm slices as described in Section 2.4. These were subjected to FTIR microscopy point analysis across a 10–15 different regions of interest across the samples surface. The spectra were collated and the position of the 5 —O stretching frequency around 1040–1050 cm⁻¹ was averaged for each sample. The effect of differing concentrations of drug loading on the stretching absorption of the sulfonate binding groups on the beads could then be evaluated.

2.6. X-ray micro-computer tomography (μ-CT) analysis for bead radiopacity

Unloaded LUMI™ and LUMI™ loaded with vandetanib at concentrations of 30, 60 and 100 mg/mL were uniformly suspended in 1% agarose solution in nunc tubes. μ-CT 3D scanning was performed to quantify individual bead radiopacity in Hounsfield units (HU) of each bead in the sample. μ-CT analysis was performed by Reading Scientific Services Ltd., Reading, UK.

2.7. Vandetanib release from beads

DC Bead™ and DC Bead LUMI™ were loaded with 20 mg/mL and 40 mg/mL of vandetanib respectively. The volume of DC Bead™ decreased by 50% following loading leading to a final dose density of 40 mg/mL. Aliquots of 0.3 mL of beads each containing 12 mg vandetanib were placed in amber jars containing 1 L of pH 7 PBS (Source Bioscience, Nottingham, UK), stirring at 200 rpm. At determined time points, stirring was stopped to allow beads to settle before removing a 5 mL sample of PBS for vandetanib quantification by HPLC as described in Section 2.2. 5 mL of fresh PBS was replaced after each timepoint.

2.8. Evaluation of suspension, handling and administration of vandetanib loaded DC Bead LUMI™

A series of evaluations were conducted to ensure that loading of vandetanib into the beads did not adversely affect their handling and delivery through a microcatheter. DC Bead LUMI™ (70–150 μm) was loaded with 100 mg/mL of vandetanib as described in Section 2.2, excess loading solution removed and the bead slurry (1 mL) mixed with contrast agent (9 mL Omnipaque 350®, GE Healthcare, Oslo, Norway). After transferring into a 20 mL Luer-lok™ syringe (BD Plastipak, Temse, Belgium) a three-way stopcock (Discofix®, B. Braun, Melsungen, Germany) was attached to the syringe and another syringe attached to enable transfer between them for suspension. Beads were transferred between two syringes in injection mixture of Omnipaque 350® and water 20 times. Suspension time was measured by the time it took for 25% of the solution to be free from beads, i.e. time taken for the top of the bead suspension to fall to 75% of the internal height of the syringe (n = 3). The suspension was passed through a 2.4 Fr catheter (Progreat®, Terumo Corp., Tokyo, Japan) and the beads assessed visually for any signs of deformation of their shape or presence of fragmentation using optical microscopy as in Section 2.3.

2.9. Stability of vandetanib loaded DC Bead LUMI™ contrast agent suspension

When used in hospital, on occasion DEBs are often prepared in the hospital pharmacy the night before and stored in a refrigerator. Drug elution from vandetanib loaded DC Bead LUMI™ (70–150 μm) suspended in contrast agent was therefore investigated by taking aliquots of the delivery media supernatant over a period of 24 h and analysing them using the HPLC method described in Section 2.2 to determine the amount of vandetanib eluted (n = 3). The chromatographic peak was also analysed to determine if there was any drug degradation during this period.

3. Results

3.1. Vandetanib loading and maximum capacity

At acidic pH, positively charged amines on vandetanib molecules interact with negatively charged sulfonate groups within the microsphere.
hydrogel network, enabling drug loading. From the theoretical prediction shown in Fig. 8, the major vandetanib microspecies, a 2+ charged form and a 1+ charged form, are predominant at pH 2.1 and pH 6.8 respectively, with a 50:50 proportion of each form at pH 4.4. Vandetanib solutions were prepared at each of these pHs and added to beads in excess to assess the effect on maximum loading capacity.

Maximum vandetanib capacity increased with pH (proportion of 1+ form) as expected, with maximum capacity at 30 mg/mL for DC Bead™ and 135 mg/mL for DC Bead LUMI™ (Fig. 1). Initial analysis of depleted loading solution from DC Bead™ showed a higher than expected loading efficiency, but with subsequent washes in water the actual loading dose was found to be lower suggesting that some drug was weakly associated with the bead surface and able to be removed with water. This effect was less pronounced with DC Bead LUMI™. Below maximum capacity, a loading efficiency of ~99% was achieved after 2 h of incubation with loading solution, suggesting fast adsorption of vandetanib into beads. When considering the respective chemical compositions of both beads, the theoretical maximum loading capacity based upon the content of sulfonic acid drug binding residues is higher for both DC Bead™ and DC Bead LUMI™ than the actual experimentally determined maximum drug binding capacity. For DC Bead, the maximum amount of drug loaded varies from 81 to 91.2% of the theoretical, compared to 74.5–80.4% for DC Bead LUMI™, both dependent upon the pH of the loading solution.

3.2. Bead morphology, size and density after loading

After loading with vandetanib, beads retained a smooth, spherical appearance (Fig. 2A–D). Loading DC Bead M1™ at maximum vandetanib capacity caused a significant decrease in mean diameter (35%, p < 0.0001) as well as a narrowing of the size distribution (Fig. 2E). The size decrease showed a trend with decreasing pH. The size reduction corresponded with a reduction in settled bead volume of over 60%. The bead density increase from 1.03 ± 0.03 g/cm³ (unloaded) to 1.15 ± 0.09 g/cm³ (loaded at 30 mg/mL), this small increase due to the decrease in size and contraction of the matrix. In contrast, the mean diameter of DC Bead LUMI™ loaded at maximum capacity did not significantly decrease at any pH (Fig. 2F), despite a 15% reduction in the settled bead volume at pH 6.8 only. The density increased only minimally from 1.29 ± 0.03 g/cm³ (unloaded) to 1.31 ± 0.09 g/cm³ when loaded at 100 mg/mL due to the marginal size change.

3.3. Vandetanib distribution in beads

Scanning electron microscopy showed the sectioned internal structure of DC Bead LUMI™ to be homogeneous and devoid of any visible pores (Fig. 3(a)). SEM-EDX analysis was used to detect vandetanib distribution in sectioned sample loaded at maximum capacity of 135 mg/mL. Bromine was selected as the element of interest for the

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Fig. 2. (A) Unloaded DC Bead™; (B) vandetanib-loaded DC Bead™ (30 mg/mL, pH 4.6); (C) unloaded DC Bead LUMI™; (D) vandetanib-loaded DC Bead LUMI™ (100 mg/mL, pH 4.6). Scale bars 100 µm. (E) DC Bead™ and (F) DC Bead LUMI™ size decrease after vandetanib loading at differing pHs.
drug distribution, being present in vandetanib molecules but not in DC Bead LUMI™. The sulfur and iodine distributions were also mapped to gain insight into the bead internal structure. Elemental mapping revealed that bromine was evenly distributed throughout the sectioned beads (Fig. 3(b)), the bromine peak seen in the EDX spectrum (arrow, Fig. 3(e)) being absent in the spectrum for control (unloaded) beads (data not shown). Sulfur and iodine distributions also appeared to be evenly distributed within the bead structure (Fig. 3(c) and (d)) (Duran et al., 2016). The table insert in Fig. 3(e) shows there is an excellent agreement between the measured weight percent of elements by EDX compared with the theoretical calculated weight percentage expected based upon 135 mg/mL loading of vandetanib into DC Bead LUMI™, the latter which possesses 177 mg/mL iodine (Duran et al., 2016).

3.4. Evaluation of drug-bead interactions using FTIR microscopy

As the dose of vandetanib loaded into DC Bead LUMI™ was increased, the frequency of the S==O stretch from the sulfonate groups of the beads was seen to shift steadily to lower wavenumber (Fig. 4).

![Fig. 3. (a) Scanning electron microscopy image of a sectioned vandetanib-loaded DC Bead LUMI™ sample; (b) bromine elemental mapping distribution image; (c) sulfur elemental mapping distribution image; (d) iodine elemental mapping distribution image; (e) EDX elemental spectrum showing bromine peak for the vandetanib loaded bead and elemental analysis (table inset).]
of vandetanib, the radiopacity is seen to have increased by 621 Houns-levell.

The amount of vandetanib released from the beads into the contrast medium 15 min after mixing was 3.3 mg, which increased to just 3.5 mg in total after 24 h storage. The drug was seen to be unaltered and remained within specification, >98.0% purity with no single impurity >0.2%. This shows that a bead suspension could be potentially prepared in advance and stored overnight without significant elution of drug into the delivery medium and without risk of drug degradation.

3.6. Vandetanib release from beads

The elution of vandetanib from DC Bead™ and DC Bead LUMI™ into PBS was analysed using an in vitro USP type II dissolution model, using 0.3 mL aliquots of beads that had been loaded at equivalent dose densities of 40 mg/mL at pH 4.4 (12 mg per aliquot). Drug release showed a rapid burst phase in the first 2 h, after which it began to plateau (Fig. 6). The total percentage release of vandetanib over the test period was higher from DC Bead™ than from DC Bead LUMI™, achieving on average 85% drug release in 24 h, compared to an average of 50% from DC Bead LUMI™.

3.7. Evaluation of suspension, handling, delivery and stability of vandetanib loaded DC Bead LUMI™

When DC Bead LUMI loaded with 100 mg of vandetanib was suspended in Omnipaque 350® (Section 2.8) the beads formed a uniform suspension and showed no signs of bead agglomeration. The bead suspension did not adhere onto the surface of the vial and was easily transferred into a syringe for delivery. The average time in suspension within the syringe was >6.5 min which is more than sufficient for practical usage. The bead suspension was delivered through a 2.4 Fr microcatheter with ease and there was no occurrence of catheter blockage during bead delivery. The beads remained spherical with no signs of fragmentation post-delivery.

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4. Discussion

Drug candidates that have been loaded into DC Bead™ have conventionally been water soluble cationic salt forms that allow for diffusion and ion-exchange into the anionically-charged hydrogel matrix of the bead (Forster et al., 2010; Guiu et al., 2015; Lewis, 2009; Lewis et al., 2007; Taylor et al., 2007). MTKis represent a group of small molecules with widely differing structures that impact drug solubility, lipophilicity and ionization potential. Different process strategies may therefore be required when considering how to load sufficient amounts of these compounds in an efficient way into the bead matrix, and subsequently what intermolecular drug-bead interactions will control the release of the molecule.

The chemical structures of a selection of MTKis considered for combining with DEBs are presented in Fig. 7. Sorafenib loading into DC Bead™ has been reported but the low solubility of the drug in aqueous media requires a loading process of several sequential sessions of loading from low drug concentration solution to achieve sufficient quantities of loaded drug (Lahti et al., 2015a). This can be overcome by use of a DMSO swelling process in which drug dissolved in DMSO can be used to swell the beads, followed by a precipitation step as the drug swollen beads are immersed in water (Forster et al., 2012). This however, leaves the drug in a microprecipitated form in which its elution is dependent upon the dissolution of the particulates. The partial ethanol solubility of sorafenib can be used to produce suitable loading solutions in ethanol:water mixtures in which the drug can be exchanged into the bead matrix. Sorafenib however, unlike, many of the other MTKis, has no protonatable amine group and relies upon strong hydrogen bonding of the amines on the urea group to interact with carboxylate residues in at its target site. This may also provide a source of interaction between drug and bead to ensure controlled release.

Sunitinib on the other hand has a pendant tertiary diethyl substitu-
ed amine that can be protonated to provide a ionic interaction with the
beads (Lahti et al., 2015b.). Solubility is improved below pH 6.8 and is further enhanced with the addition of a sugar or polyol additive to aid loading (Fuchs et al., 2015). Vandetanib, erlotinib, gefitinib and dasatinib all share a common secondary amine bridging function attached to a pyrimidine ring, providing additional sites of ionization to aid in ion-exchange and increase the solubility of these compounds in water at reduced pH. The positive charge residing on this aromatic portion of the molecule however, can be significantly delocalised across the conjugated ring system, which lessens its capability for charge-charge interaction (Lewis, 2009).

Based on the theoretical ionization of vandetanib in solution at different pH (Fig. 8) and therefore the relative concentrations of different vandetanib charge forms, the drug loading maximum capacity should be influenced by the pH of the loading solution, which has been confirmed in Fig. 1. For both DC Bead™ and DC Bead LUMI™, there was a positive relationship between pH of the loading solution and the maximum amount of vandetanib that could be loaded into the beads. This is due to the fact that as pH decreases, a greater proportion of vandetanib molecules in solution have 2 positive charges, and can therefore occupy 2 sulfonate binding sites within the beads, shifting towards a 2:1 binding ratio. Regardless of the pH, neither bead was able to load the theoretical maximum drug loading based upon sulfonic acid group content, although DC Bead™ was seen to load closer to the theoretical maximum (91.2%) than DC Bead LUMI™ (80.4%), the latter having a denser, more hydrophobic gel matrix in which access to all binding sites may be more restricted.

In DC Bead™, a slight decrease in size range was observed with decreasing pH. This is in keeping with previously observed effects of loading the antineoplastic mitoxantrone, which has 2 positively charged sites, and thus exerts an increased shrinking effect on DC Bead™ which is suggested to be an effect of ‘pulling’ the hydrogel network closer together by interacting with multiple sites (Lewis, 2009). The drug-bead charge-charge interaction through the sulfonate groups was confirmed by the shift of the S =O stretching frequency to lower wavenumber with increasing drug dose, a phenomenon also observed for both doxorubicin and irinotecan binding to DC Bead™ (Lewis and Dreher, 2012; Namur, 2009). Interestingly, when 60 mg/mL loading was performed at pH 2.1, 4.4 and 6.8, the S =O frequency was seen not to change significantly, even though at this dose the DC Bead LUMI™ is only at about 50% binding capacity. At pH 2.1 it is expected that vandetanib would carry two positive charges and hence capable of interaction with nearly all binding sites in the beads. The second charge residing on the conjugated ring system would not be expected to interact as strongly as that on the piperidine ring as indicated by the lack of change in the S =O stretching frequency.

Furthermore, although it could be expected that vandetanib molecules binding to 2 sites may be released more slowly in an elution model, the release profile of vandetanib from DC Bead LUMI™ did not vary significantly depending on the pH of the loading solution (data not shown). Possible reasons for this are that the ionic interactions are not strong enough to be affected in this manner, or perhaps the neutral pH of the elution medium negates this pH dependent effect as soon as the ion exchange process begins.

Release of vandetanib from DC Bead LUMI™ was incomplete, despite maximum concentrations reached in the elution vessel remaining lower than the theoretical solubility of 0.008 mg/mL (Fig. 6A). The higher
density and therefore less ‘open’ structure of DC Bead LUMI™ likely limits movement of drug molecules out of the beads. However, by adjusting the pH of the PBS elution medium to 5, vandetanib release rate from LUMI™ was slightly improved with total drug release increasing by 10% to 60% (data not shown). Hypoxic tumours are known to be acidic due to production of lactic acid by anaerobic glycolysis. Moreover efficient arterial embolization will impede clearance of lactic acid from tumoural tissues, contributing to the acidic microenvironment (Chao et al., 2016), which may somewhat facilitate release in the case of acidic-soluble drugs. Similarly to doxorubicin (Hecq et al., 2013), vandetanib binding to DEB has been shown to be fully reversible as the total dose can be extracted using a solution of potassium chloride and ethanol. Using improved elution models with open-loop flow through conditions as described by Swaine et al. may facilitate the modelling of full extended vandetanib release by preventing saturation of elution medium and more closely mimicking the in vivo situation (Swaine et al., 2016).

The addition of the iodinated moiety that renders DC Bead LUMI™ radiopaque increases the solid content of the beads (Duran et al., 2016) and introduces hydrophobic portions to the bead structure which may interact with various drugs thereby altering drug loading and release properties. Increasing loading of vandetanib also leads to a proportional increase in the radiopacity of the DC Bead LUMI™ (Fig. 4) which is not attributable to a densification of the internal structure as the mean diameter of the beads are essentially unchanged with drug loading (Fig. 2F). The existence of a bromine atom on the vandetanib structure is the likely source of the added radiopacity, which increases by approximately 25–26 Hounsfield units per mg of radiodense atom (iodine and bromine) present.

The data presented in this study has confirmed that DC Bead LUMI™ has desirable physicochemical characteristics suitable for use as an anti-angiogenic DEB. Firstly, maximum vandetanib loading capacity is high, reaching maximum doses of over 120 mg/mL at pH 6.8 and even when decreasing pH to maintain vandetanib solubility doses of 100 mg/mL are still feasible. The increased solid content leads to a higher density of drug binding sites than standard DC Bead™. This increased capacity may compensate for the slower drug release rate. Secondly, DC Bead LUMI™ was more resistant to size changes after loading with vandetanib, compared with DC Bead™ which had a significant reduction in bead size and volume. Although there has been a trend towards using smaller size beads in recent years (Lencioni et al., 2012), it has been observed that particles of around 40 μm pose a risk of potentially fatal complications resulting from off-target embolisation (Bonomo et al., 2010; Brown, 2004). The radiopacity of DC Bead LUMI™ may also provide advantages in this respect due to the potential for intra and post-procedural feedback on bead delivery (Duran et al., 2016). Finally, loading of the drug did not adversely affect the properties of the beads in terms of their suspension, handling and delivery in contrast agent. When mixed into suspension only a small amount of drug (3.5 mg) was released into Omnipaque contrast agent and the drug was shown to be stable in this mixture over the 24 h storage condition selected for the test. These preliminary evaluations confirm that
vandetanib loaded DC Bead LUMI™ performs in a manner amenable for a DEB-TACE procedure.

Recent investigations have been performed using DC Bead™ loaded with the VEGFR inhibitor sunitinib (Fuchs et al., 2015; Fuchs et al., 2014), which have shown significant effects on endothelial cell proliferation, apoptosis and migration, with both sunitinib beads and free sunitinib having similar activity in vitro. Sunitinib beads had modest anti-proliferative effects against a range of cancer cell lines, with lack of direct cytotoxicity due to the mechanism of action of sunitinib being VEGFR inhibition. As well as inhibiting VEGFR, vandetanib also targets EGFR and therefore has the potential to also directly impede cancer cell proliferation and induce apoptosis. In vitro assays have revealed that vandetanib has a significant anti-proliferative effect on endothelial cells and induces apoptosis in hepatoma cell lines at a concentration of < 10 μmol/L (4.75 mg/ml) (Inoue et al., 2012). The elution studies performed here indicate that the release of vandetanib levels in excess of this concentration is feasible. In support of this preliminary data we have recently reported on a safety and pharmacokinetics study of vandetanib DEB-TACE in healthy swine liver which demonstrated that the treatment was well tolerated and confirmed the presence of therapeutic concentrations of vandetanib up to 30 days after administration (Cuzzuzman et al., 2016).

5. Conclusions

Vandetanib can be efficiently loaded and released from DC Bead™ and DC Bead LUMI™ whilst maintaining microsphere integrity and properties suitable for use as an embolization agent. The inclusion of vandetanib in a DEB warrants further characterisation in vitro and in vivo to determine its efficacy in models of HCC.

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References


