LABORATORY INVESTIGATION

An In Vitro Evaluation of Four Types of Drug-Eluting Microspheres Loaded with Doxorubicin

Thierry de Baere, MD, Stephen Plotkin, BS, Renee Yu, MS, Allison Sutter, BS, Yue Wu, PhD, and Gregory M. Cruise, PhD

ABSTRACT

Purpose: To compare in vitro properties of 4 drug-eluting embolic agents loaded with doxorubicin.

Materials and Methods: DC Bead (100–300 μm), LifePearl (200 μm), HepaSphere (30–60 μm), and Tandem (100 μm) microspheres were loaded with 40 mg/20 mL of doxorubicin per milliliter of microspheres. Loading, elution, diameter changes after loading, changes in the amount of doxorubicin loaded over 2 weeks in storage, and time in suspension were evaluated.

Results: All microspheres loaded > 99% doxorubicin within 1 hour. In vitro elution reached a plateau by 6 hours, with 30% ± 5, 21% ± 2, 8% ± 3, and 6% ± 0 of the loaded doxorubicin eluted for LifePearl, DC Bead, HepaSphere, and Tandem microspheres, respectively, with at least 1 statistically significant difference between at least 2 of the products in doxorubicin eluted at every time point. The times to elute 75% of the total released doxorubicin were 197, 139, 110, and 77 min for DC Bead, LifePearl, HepaSphere, and Tandem microspheres, respectively. The average diameters of LifePearl, DC Bead, and Tandem microspheres were reduced after loading by 24%, 20%, and 9%, respectively. After suspension in contrast medium, no changes were observed in doxorubicin loading over 2 wk. After loading, times in suspension were 8.4 min ± 0.2, 6.0 min ± 0.1, 3.1 min ± 0.2, and 2.9 min ± 0.3 for Tandem, LifePearl, DC Bead, and HepaSphere microspheres, respectively.

Conclusions: Although drug-eluting embolic agents universally loaded doxorubicin within 1 hour, the elution amounts, rates of release, diameter shrinkage, and times in suspension varied by product.

ABBREVIATION

HPLC = high performance liquid chromatography

A randomized clinical trial evaluating transarterial chemoembolization for the treatment of intermediate-stage hepatocellular carcinoma (1) showed the benefit of doxorubicin-loaded drug-eluting embolic agents compared with transarterial chemoembolization with Lipiodol (Guerbet, Villepinte, France). Currently, various types of drug-eluting embolic agents are commercially available for use with doxorubicin, including DC Bead (BTG, Farnham, United Kingdom), HepaSphere (Merit Medical, South Jordan, Utah), LifePearl (Terumo European Interventional Systems, Leuven, Belgium), and Tandem (Boston Scientific, Marlborough, Massachusetts) microspheres. DC Bead microspheres consist of a polyvinyl alcohol hydrogel modified with sulfonate groups (2). HepaSphere microspheres consist of a poly(vinyl alcohol-co-sodium acrylate) hydrogel (3). LifePearl microspheres consist of a hydrogel network of poly (ethylene glycol) and 3-sulfopropyl acrylate. Tandem microspheres consist of a hydrogel core made of sodium poly(methacrylate) and an outer biocompatible shell of poly(bis[trifluoroethoxy]phosphazene) (4). For all four types of microspheres, the drug loading mechanism is the ionic interaction of the cationic doxorubicin with the anionic functional groups of the microspheres.

Despite the clinical use of several types of doxorubicin-loaded microspheres, a systematic analysis of the similarities

From the Department of Interventional Radiology (T.d.B.), Gustave Roussy Cancer Center, 114 rue Edouard Vaillant, 94 805 Villejuif, France; Unité de formation et de recherche (UFR) Médecine Le Kremlin-Bicêtre (T.d.B.), Universite Paris-Sud XI, Le Kremlin Bicêtre, France; and MicroVention (S.P., R.Y., A.S., Y.W., G.M.C.), Tustin, California. Received March 4, 2016; final revision received May 11, 2016; accepted May 12, 2016. **Address correspondence to** T.d.B.; E-mail: thierry.debaere@gustaveroussy.fr

T.d.B is a paid consultant for Terumo (Tokyo, Japan). S.P., R.Y., A.S., Y.W., and G.M.C. are paid employees of Terumo.

J Vasc Interv Radiol 2016; XX:▮▮▮–▮▮▮

http://dx.doi.org/10.1016/j.jvir.2016.05.015

and differences of the commercially available products is lacking. The in vitro characteristics of DC Bead and HepaSphere microspheres loaded with doxorubicin have been reported (2,5); however, the characteristics of LifePearl and Tandem microspheres loaded with doxorubicin have not been reported to our awareness. As such, the purpose of the present study was to compare in vitro properties of DC Bead, HepaSphere, LifePearl, and Tandem microspheres loaded with doxorubicin.

MATERIALS AND METHODS

Materials

Microspheres evaluated in this study included DC Bead (100–300 μm), HepaSphere (30–60 μm dry and 120–240 μm expanded), LifePearl (200 μm), and Tandem (100 μm) microspheres. Generic doxorubicin hydrochloride (Pfizer, New York, New York) at a concentration of 2 mg/mL and nonionic Omnipaque (GE Healthcare, Princeton, New Jersey) 300 mgI/mL were used in this study.

Doxorubicin Loading, Elution, and Loading Stability

Each package of microspheres, containing 2 mL of microspheres, was split into two equal samples to form 1-mL aliquots. Doxorubicin loading of five aliquots of each product was then evaluated in accordance with the manufacturers' instructions. The excess supernatant was removed, and 20 mL of doxorubicin (40 mg) solution was added. The microspheres were agitated on a rocker during the incubation. At 0.25, 0.5, 1, 2, 3, 4, 5, and 24 hours of incubation, 50 μL of the solution was removed and diluted with 1.0 mL of water, and the concentration of doxorubicin was quantified by using a validated high-performance liquid chromatography (HPLC) method. Briefly, doxorubicin concentration was quantified by using an Agilent 1260 Infinity HPLC system with a Phenomenex Gemini-NX 3-μm C18 column (4.6 mm × 50 mm) from 5 to 400 ppm. The mobile phase was 73:27 15 mM $NH_4OH/(NH_4)3PO_4$ (pH 10):acetonitrile at a rate of 1 mL/min. The injection volume was 10 μL, and the wavelength was 234 nm. Mass and percentage loading were calculated from the concentration data.

The diameters of unloaded and doxorubicin-loaded microspheres were measured by using an AxioZoom V.16 motorized stereo zoom microscope with a Plan NEOFLUAR Z 1×/0.25 free working distance 56 mm objective and an AxioCam high-resolution cooled camera (Zeiss, Thornwood, New York). Diameter measurements were calculated by using AxioVision SE64 measurement software (Zeiss). For each measurement group, the diameter of at least 200 μm was determined.

For determination of elution, doxorubicin-loaded microspheres were placed in one of seven flow cells of a CE 7smart USP 4 system (Sotax, Westborough, Massachusetts). Five replicates per microsphere group were evaluated. The Sotax elution system was prepared with a 500-mL sink of 0.9% saline solution per channel at a temperature of 37°C and a flow rate of 8.0 mL/min. Samples, 1 mL per time point, were taken at 0.33, 0.67, 1, 2, 3, 4, 5, 6, and 24 hours after the beginning of the elution. Doxorubicin concentration was determined by using the validated HPLC method described earlier. Mass and percentage elution were calculated from the concentration data.

For determination of stability of the loading over time in storage, doxorubicin-loaded microspheres were placed in 65:35 (volume/volume) contrast agent:water for injection solution or 100% contrast agent. Five replicates per microsphere group were evaluated per aqueous solution. After loading, microspheres were placed in 10 mL of the solution and stored at 4°C. At periodic time points from 1 hour to 10 days, samples were collected and analyzed by using a validated HPLC method with a range from 5 to 400 ppm to quantify the doxorubicin in the fluid. Briefly, with the use of the same equipment and column described earlier, a gradient method with the same mobile phases was used. The injection volume was 10 μL. The mobile phase flow rate was 1 mL/min with 15%–33% acetonitrile from 0 to 2.5 minutes and 33% acetonitrile from 2.5 to 2.6 minutes. Mass and percentage elution were calculated from the concentration data.

Time in Suspension

Time in suspension was evaluated for doxorubicin-loaded microspheres in a manner previously described (6). Six replicates per microsphere group were evaluated. After loading, the supernatant was expressed from the microspheres and replaced with 8 mL of 50:50 Omnipaque 300:water for injection (volume/volume) solution in a 10-mL syringe. The microspheres and contrast agent:water solution was mixed by 15 passes of syringe-to-syringe mixing. At the end of the mixing, all the contents were placed in one syringe, and that syringe was immediately placed vertically on a countertop. The time taken for the microspheres to vacate one third of the syringe was taken as the time in suspension.

Statistical Analysis

Statistical analyses were performed by using Minitab 17 (Minitab, State College, Pennsylvania). Differences in continuous data were assessed by using a one-way Welch analysis of variance. If the analysis of variance indicated a significant difference, a two-tailed t test was performed to identify the specific differences. Statistical significance was accepted at $\alpha \leq 0.05$.

RESULTS

Doxorubicin Loading, Elution, and Loading Stability

All four microsphere types quickly and repeatedly loaded doxorubicin (Fig 1). HepaSphere microspheres