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Research Article

Effect of Vial shaking on Loading Time of Epirubicin into Drug-Eluting Bead

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ABSTRACT

Background: Although DC Bead has been useful in treatment of multiple and large hepatocellular carcinoma, loading time of doxorubicin into the DC Bead takes a long time of 30-120 minutes. Epirubicin is also used as an antitumor agent together with DC Bead, but its loading efficiency was not sufficiently elucidated. Methods: To shorten loading time of epirubicin into DC Bead (100-300μm, 300-500μm, 500-700μm), we examined the following three methods after mixing the drug: (a) let stand in room temperature, (b) agitated for 30 seconds with Vortex mixer, and (c) sonicated for 30 seconds with ultrasonic cleaner. After loading of epirubicin by each method, supernatant concentration for epirubicin was assayed at 5, 10, 30, 60, and 120 minutes. Results: Epirubicin loading rates for small bead (100-300μm) at 5 minutes were 82.9 % in group a, 93.8% in group b, and 79.9 % in group c. Similarly, medium bead (300-500μm), 40.1% in group a, 65.7% in group b and 45.5% in group c, respectively. In large-sized bead (500-700μm), loaded rates of epirubicin were 38.8% in group a, 59.0% in group b and 48.0% in group c. Agitation of mixture of epirubicin and DC Bead with Vortex mixer significantly shortened the loading time, but sonication did not affect the time required. Microscopic examination did not lead to any morphological change of microspheres in all the methods. Conclusions: Short time of agitation with Vortex mixer reduced the necessary time for loading of epirubicin in every standard of DC Bead.

Keywords: epirubicin, DC Bead, Drug eluting bead, transcatheter arterial embolization, hepatocellular carcinoma, load

INTRODUCTION

Since hepatic resection and percutaneous radiofrequency ablation are applicable in only 30–40% of patients with hepatocellular carcinoma (HCC), transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with intermediately advanced HCC[1,2]. Various procedures and tools for more effective TACE have been developed recently. In the last decade, drug-eluting beads for the transcatheter arterial treatment of HCC have been developed to actively loaded doxorubicin from its solution and to release it in a controlled and prolonged fashion in the tumor's arterial network[3-6]. Among them, DC Bead™ (Biocompatibles, UK) has been designed to deliver a higher and more sustained release of drug directly into the tumor and a low release of drug into the systemic circulation, with the intention to maximize the drug's effectiveness in terms of response, while significantly reducing its systemic toxicity. The microspheres contain anionic groups in their chemical structures which may interact with positively charged parts of anthracyclines by an ion-exchange mechanism, leading to controlled and sustained intra-tumoral drug retention in the treatment of HCC.

In Japan, epirubicin (Farmorubicin™, Pfizer Japan, Tokyo, Japan) is commonly used instead of doxorubicin to load to DC Bead, because of cardiac side effect of doxorubicin[7,8]. However, loadability of epirubicin into DC Bead was not sufficiently elucidated, and it is also not examined how to mix and load epirubicin into DC Bead efficiently. In vitro study, it requires 20 to 60 min for DC Bead to incorporate at least 90% of 50-75mg of doxorubicin when DC Bead mixture is let stand after addition of the drug solution, with the time varying according to microsphere size[9]. However, it is too long to wait during angiographic procedure in actual clinical setting.

The purpose of this study is, therefore, to find appropriate procedure to shorten the loading time of epirubicin into DC Bead.

MATERIALS AND METHODS

Embolic Material

DC Bead™ (Biocompatibles UK Ltd, Farnham, UK) embolization microsphere is an embolic drug-eluting bead capable of loading and releasing a controlled manner chemotherapeutic agent. It is produced from a biocompatible polyvinyl alcohol hydrogel that has

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Table 1 Epirubicin concentration in supernatant of DC Bead solution (μg/ml).

Small DC Bead (100-300)	Time (min)				
	5	10	30	60	120
(a) Left at room temperature	2131.81	2042.32	1908.32	1529.73	1478.84
(b) Vortex mixer	776.30	866.62	808.26	765.49	643.26
(c) Ultrasonic cleaner	2508.76	2463.84	2393.05	2284.65	2127.40
Medium DC Bead (300-500)	Time (min)				
	5	10	30	60	120
(a) Left at room temperature	7487.97	6250.58	5930.49	5742.28	5656.08
(b) Vortex mixer	4288.31	4062.74	3560.54	2966.27	2351.22
(c) Ultrasonic cleaner	6818.08	6681.25	5956.45	5554.85	5002.49
Large DC Bead (500-700)	Time (min)				
	5	10	30	60	120
(a) Left at room temperature	7652.10	6789.08	5107.69	4061.69	2697.50
(b) Vortex mixer	5118.82	4984.00	4197.48	3538.98	2771.81
(c) Ultrasonic cleaner	6506.06	5829.92	5123.74	4378.73	3423.05

Table 2 Calculated rates of loaded epirubicin proportion in DC Bead (percent).

Small DC Bead (100-300)	Time (min)				
	5	10	30	60	120
(a) Left at room temperature	82.9	83.4	84.7	87.8	88.2
(b) Vortex mixer	93.8	93.1	93.5	93.9	94.9
(c) Ultrasonic cleaner	79.9	80.3	80.9	81.7	83.0
Medium DC Bead (300-500)	Time (min)				
	5	10	30	60	120
(a) Left at room temperature	40.1	50.0	52.3	54.1	54.8
(b) Vortex mixer	65.7	67.5	71.2	76.3	81.2
(c) Ultrasonic cleaner	45.5	46.6	52.3	55.6	60.0
Large DC Bead (500-700)	Time (min)				
	5	10	30	60	120
(a) Left at room temperature	38.8	45.7	59.1	67.5	78.4
(b) Vortex mixer	59.0	60.1	66.4	71.7	77.8
(c) Ultrasonic cleaner	48.0	53.4	59.0	65.0	72.6

modified with sulphonate groups for the controlled loading and delivery of chemotherapeutic drugs, primarily anthracyclines. DC Bead™ was supplied in sterile vials as 2 mL hydrated bead volume in 6 mL of phosphate-buffered saline (PBS). Among four standards of DC Bead, we examined three standards of bead sizes available in Japan: 100-300μm, 300-500μm, and 500-700μm.

Antitumor agent and loading into DC Bead

Epirubicin (Farmorubicin™, Pfizer Japan, Tokyo, Japan) is a highly water-soluble anti-tumor agent used for the treatment of HCC. A total of 50mg of epirubicin was reconstituted in 2 mL of water for injection (Otsuka Pharmaceutical, Tokyo, Japan).

Six mL of the phosphate buffer saline was removed from a vial of DC Bead, and 2 mL of reconstituted epirubicin solution was added to the vial. Subsequently, the DC Bead with epirubicin solution were (a) let stand in room temperature, (b) agitated for 30 seconds with Vortex mixer (VORTEX-GENIE 2 SCIENTIFIC, Scientific Industries, Philadelphia, USA), and (c) sonicated for 30 seconds with ultrasonic cleaner (US-1R, As One Co. Ltd, Osaka, Japan) using 42 kHz ultrasound generator (Figure 1). Agitation or sonication was performed for a period of 30 seconds, since the microparticles formed aggregates when performed for longer than one minute. In all the four manners of epirubicin loading, we examined three times to confirm the reproducibility for each procedure.

Assessment of epirubicin loading

The epirubicin loading into DC Bead was assessed at 5, 10, 15, 20, 30, 60, and 120 min after addition of the drug solution to DC Bead for each condition of the mixtures (methods a, b, and c). The amount of loaded epirubicin was calculated from the residual unloaded drug content in the solution: the extent of drug uptake into DC Bead was estimated by measuring the depleted epirubicin content in the supernatant of the mixture using a spectrophotometer (ZA3000, Hitachi High Technologies, Tokyo, Japan) with detection at a wavelength of 483 nm. In all the three manners of epirubicin loading (a, b, and c), supernatant concentration of epirubicin was measured three times, and the averages of epirubicin concentrations were applied to make the drug loading profiles.

Morphology of microspheres

Morphological assessment was performed at 30 minutes after loading using stereoscopic microscope (VHX-1000, KEYENCE Japan, Osaka, Japan) for all the standard of DC Bead in the four procedures of epirubicin loading: (a) let stand in room temperature, (b) agitated with Vortex mixer, or (c) sonicated with ultrasonic vibrator,.

RESULTS

a. Let stand at room temperature

b. Agitated with Vortex mixer for 30 sec.

c. Sonicated for 30 sec.

Figure 1: Loading procedures of epirubicin into DC Bead. Mixtures of epirubicin solution and DC Bead were (a) let stand at room temperature, (b) agitated for 30 seconds with Vortex mixer, and (c) sonicated for 30 seconds with ultrasonic cleaner.

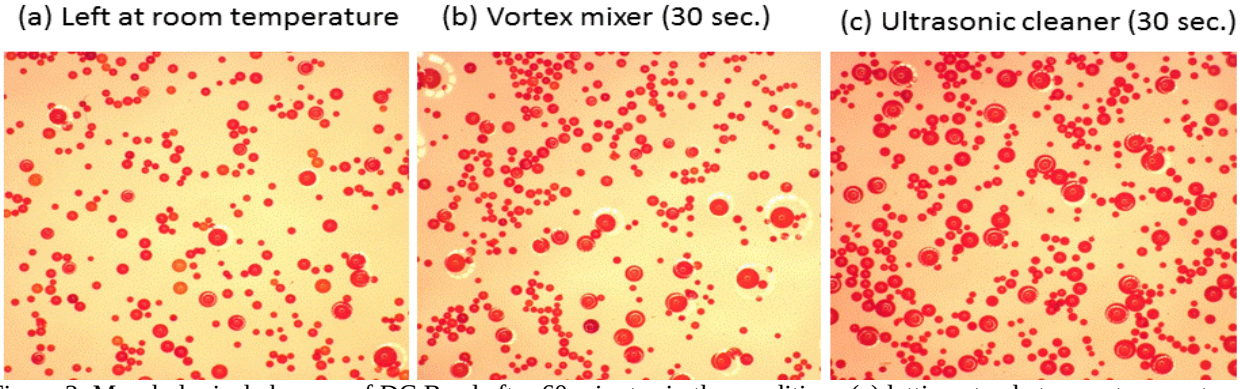

Figure 2: Morphological changes of DC Bead after 60 minutes in the conditions (a) letting stand at room temperature, (b) agitation for 30 seconds with Vortex mixer, and (c) sonication for 30 seconds with ultrasonic cleaner. Agitation and sonication did not affect microscopic finding at all.

Morphology of microspheres after loading epirubicin

Agitation and sonication of DC Bead suspension did not affect microsphere structure so long as the above procedures were applied within one minute (Figure 2a, 2b, and 2c). However, aggregated microspheres were partly found after agitation or sonication for a long time of one minute or more).

Therefore, the duration of agitation or sonication was set to be 30 seconds in the following experiments. Warming to 40°C did not cause any changes in morphology of the microsphere.

Epirubicin loading in ordinary manner at room temperature

Serial assay of supernatant concentration of epirubicin in DC Bead suspension was performed in a standard loading manner with letting stand at room temperature (procedure a)(Table 1). For a small-sized microsphere (100-300μm), average supernatant concentrations of epirubicin were 2131.81μg/ml at 5 minutes, 2042.32μg/ml at 10 min., 1908.32μg/ml at 30min., 1529.73μg/ml at 60min., and 1478.84μg/ml at 120min., respectively. Similarly for a medium-sized microsphere (300-500μm), average concentrations of epirubicin were 7487.97μg/ml at 5 minutes, 6250.58μg/ml at 10 min., 5930.49μg/ml at 30min., 5742.28μg/ml at 60min., and 5656.08μg/ml at 120min., respectively. For a large-sized one (500-700μm), 7652.10μg/ml at 5 minutes, 6789.08μg/ml at 10 min., 5107.69μg/ml at 30min., 4061.69μg/ml at 60min., and 2697.50μg/ml at 120min., respectively.

Since loaded part of epirubicin into DC Bead was considered as just subtracted amount of supernatant epirubicin, the rates of loaded proportion of epirubicin was calculated (Table 2). For a small-sized microsphere (100-300μm), average rates of loaded epirubicin were 82.9% at 5 minutes, 83.4% at 10 min., 84.7% at 30min., 87.8% at 60min., and 88.2% at 120min., respectively. Similarly for a medium-sized microsphere (300-500μm), average rates of loaded epirubicin were 40.1% at 5 minutes, 50.0% at 10 min., 52.3% at 30min., 54.1% at 60min., and 54.8% at 120min., respectively. For a large-sized one (500-700μm), 38.8% at 5 minutes, 45.7% at 10 min., 59.1% at 30min., 67.5% at 60min., and 78.4% at 120min., respectively.

The time required for epirubicin incorporation to DC Bead was shorter in the small-sized beads than in the medium- and large-sized beads.

Epirubicin loading rate into small-sized DC Bead

In the condition of Vortex mixing (procedure b), the epirubicin concentrations remained in the supernatant of the microsphere solution were 776.30μg/ml at 5 minutes, 866.62μg/ml at 10 min., 808.26μg/ml at 30min., 765.49μg/ml at 60min., and 643.26μg/ml at 120min., respectively. Vortex mixing significantly enhanced incorporation of epirubicin in a short time.

In the condition of sonication (procedure c), the epirubicin concentrations remained in the supernatant of the microsphere solution were 2508.76μg/ml at 5 minutes, 2463.84μg/ml at 10 min., 2393.05μg/ml at 30min.,

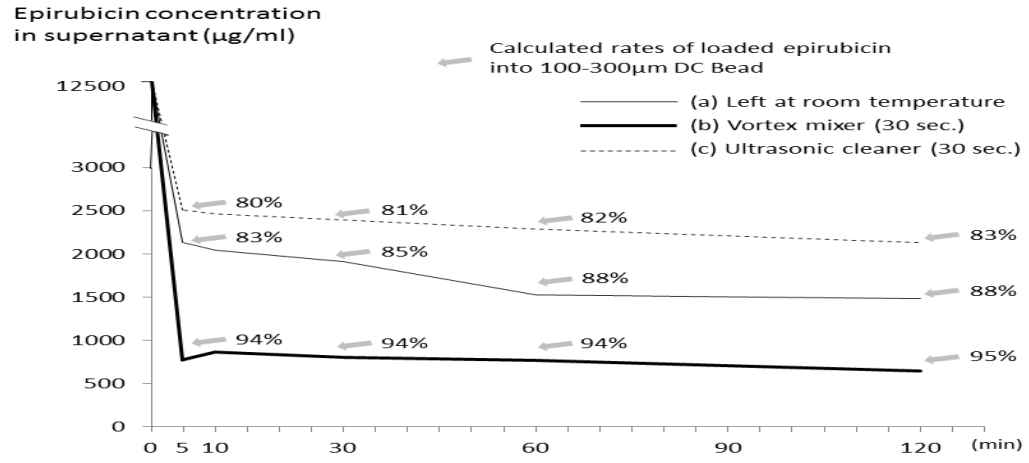

Figure 3 Incorporation of epirubicin in small-sized DC Bead (100-300μm) in the conditions of (a) letting stand at room temperature, (b) agitation for 30 seconds with Vortex mixer, and (c) sonication for 30 seconds with ultrasonic cleaner. Vortex mixing significantly promoted the process of epirubicin loading with a rapid incorporation of 94% after 5 minutes.

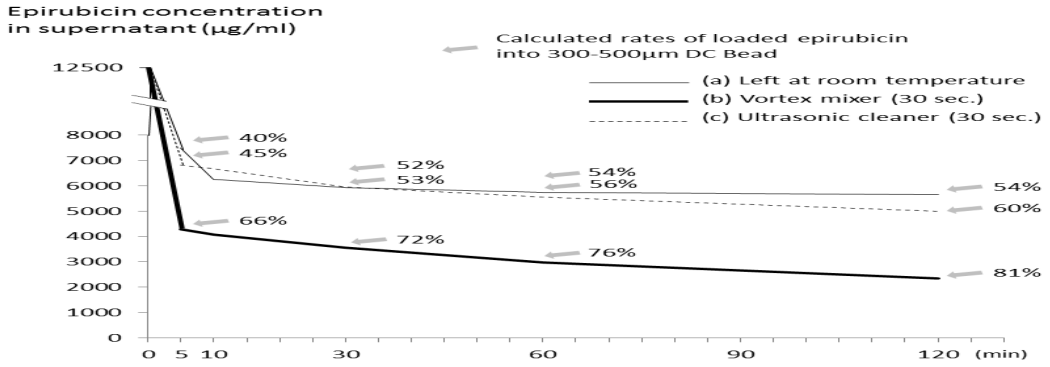

Figure 4 Incorporation of epirubicin in medium-sized DC Bead (300-500μm) in the conditions of (a) letting stand at room temperature, (b) agitation for 30 seconds with Vortex mixer, and (c) sonication for 30 seconds with ultrasonic cleaner. Vortex mixing significantly shortened the process of epirubicin loading with incorporation of 66% after 5 minutes.

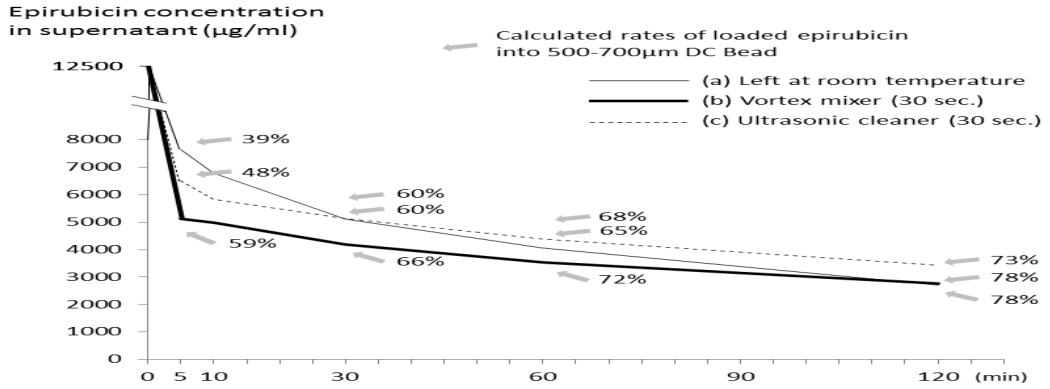

Figure 5 Process of epirubicin loading in large-sized DC Bead (500-700μm) in the conditions of (a) letting stand at room temperature, (b) agitation for 30 seconds with Vortex mixer, and (c) sonication for 30 seconds with ultrasonic cleaner. Vortex mixing slightly enhanced the epirubicin loading with 59% after 5 minutes.

2284.65μg/ml at 60min., and 2127.40μg/ml at 120min., respectively. Ultrasonic treatment did not affect the incorporation of epirubicin into the microsphere (Table 1).

Since loaded part of epirubicin into DC Bead was considered as just subtracted amount of supernatant epirubicin (Table 2), calculated loaded amounts of epirubicin were drawn in the conditions a, b, and c (Figure 3). Loaded rates were significantly higher in Vortex mixing group than the other procedures. Epirubicin loading rates at 5 minutes were 82.9 % in procedure a (left at room temperature), 93.8% in procedure b (Vortex mixing), and 79.9 % in procedure c (sonication).

Epirubicin loading rate into medium-sized DC Bead

In the condition of Vortex mixing (procedure b), the epirubicin concentrations in the supernatant were 4288.31μg/ml at 5 minutes,4062.74μg/ml at 10 min., 3560.54μg/ml at 30min., 2966.27μg/ml at 60min., and2351.22μg/ml at 120min., respectively. Vortex mixing significantly enhanced incorporation of epirubicin in a short time.

In the condition of sonication (procedure c), the epirubicin concentrations were 6818.08μg/ml at 5 minutes, 6681.25μg/ml at 10 min., 5956.45μg/ml at 30min., 5554.85μg/ml at 60min., and 5002.49μg/ml at 120min., respectively. Ultrasonic treatment did not affect the incorporation of epirubicin (Table 1).

Calculated loaded amounts of epirubicin were drawn in the conditions a, b, and c (Figure 4). Loaded rates were significantly higher in Vortex mixing group than the other procedures. Epirubicin loading rates at 5 minutes were 40.1 % in procedure a (left at room temperature), 65.7% in procedure b (Vortex mixing), and 45.5% in procedure c (sonication) (Table 2).

Epirubicin loading rate into large-sized DC Bead

In the condition of Vortex mixing (procedure b), the epirubicin concentrations in the supernatant were 5118.82μg/ml at 5 minutes, 4984.00μg/ml at 10 min., 4197.48μg/ml at 30min., 3538.98μg/ml at 60min., and 2771.81μg/ml at 120min., respectively. Vortex mixing significantly enhanced incorporation of epirubicin in a short time.

In the condition of sonication (procedure c), the epirubicin concentrations were 6506.06μg/ml at 5 minutes, 5829.92μg/ml at 10 min., 5123.74μg/ml at 30min., 4378.73μg/ml at 60min., and 3423.05μg/ml at 120min., respectively. Ultrasonic treatment did not affect the efficiency of incorporation of epirubicin (Table 1).

Calculated loaded amounts of epirubicin were drawn in the conditions a, b, and c (Figure 5). Loaded rates were higher in Vortex mixing group than the other procedures. Epirubicin loading rates at 5 minutes were 38.8 % in procedure a (left at room temperature), 59.0% in procedure b (Vortex mixing), and 48.0% in procedure c (sonication) (Table 2).

DISCUSSION

TACE is widely recommended for intermediately-advanced HCC, multiple nodules and/or a large tumor difficult to resect, according to any guidelines from the Japan Society of Hepatology[10], American Association of Study of Liver Disease[11], and European Association of Study of Liver Disease[12].

Aside from the property of calibrated, targeted, and more complete occlusion of peripheral tumor vessels, drug eluting beads were recently developed to deliver drugs directly to tumor tissue to obtain sustained release of antitumor agents, and thereby improve the clinical outcomes of patients with intermediate stage of HCC with less systemic toxicity. Of drug eluting beads, DC Bead is composed of a polyvinyl alcohol polymer that modifies with sulfonate groups to form a hydrogel of high water content (>95%) and the negatively charged sulfonate interacts with the positively charged protonated amine groups of doxorubicin hydrochloride by an ion exchange process driven by entropic release of the smaller counter ions from the hydrogel[13]. Preclinical and clinical studies have demonstrated a higher and prolonged retention of doxorubicin within the tumor after TACE with DC Bead, and lower systemic plasma levels of doxorubicin, compared to cTACE[9,14-19]. A randomized controlled trial, PRECISION V study demonstrated that TACE using DC Bead had a higher response rate than conventional TACE using lipiodol and varied embolic substances but statistical difference was not obtained between the two arms[4].

There is no standard method for the choosing of drug in TACE, and actual comparisons of treatment effect are not sufficient among cisplatin, epirubicin, mitomycin, and miriplatin. In the TACE treatment of HCC in Japan, anthracyclines are commonly used at the first time of TACE together with lipiodol. The optimal combination of the particle size and dose of epirubicin was not elucidated, and ideal releasing time of epirubicin from DC Bead remained to be examined. The DC Bead and doxorubicin preparations are prepared by the addition of the required amount of doxorubicin into the DC Bead by the physician prior to use. Typically the nominal doses of doxorubicin loaded per vial of DC bead will be 50mg and 75mg. This is equivalent to a nominal doxorubicin dose of 25mg and 37.5mg per 1mL of beads.

Biocompatibles Co. Ltd., the manufacturer of DC Bead, published the absorbing efficiency of doxorubicin into DC Bead and required times for 90% or 98% of certain dose of doxorubicin in each size of microsphere. Although the loading time largely depended on the size of microspheres, it required at least for 30 minutes for 90% and 60 minutes for 98% of doxorubicin, respectively. In real clinical setting, 30 to 60 minutes seems too long for usual radiologists or hepatologists to wait the preparation of doxorubicin containing microspheres at an angiographic room. To avoid the waste of time, certain radiologists order the regimen of doxorubicin in DC Bead to their hospital pharmacy a few hours prior to angiographic procedures. But DEB-TACE sometimes cannot be performed due to varied reasons including arterio-portal or arterio-venous shunting, difficulties in catheterization for parasitic feeders, unpredictable arterial spasm, and so on. Angiographic morphology of an HCC

nodule possibly proves to be unsuitable for DEB-TACE and conventional TACE is eventually chosen for the treatment. Both the waste of time and the waste of medical resources should be taken into account in an appropriate selection of treatment from the viewpoints of cost-effectiveness.

We therefore tried to shorten the loading time of an anthracycline, epirubicin into DC Bead of various standards of diameter. In clinical angiography room, vortex mixing of DC Bead solution was quick and very concise during TACE procedure. Ninety-four percent of 50mg of epirubicin was incorporated in 2ml of small-sized DC Bead with the procedure of only 30 seconds agitation with vortex mixing followed by 5 minutes in room temperature. Our results will enable the interventional radiologist to load epirubicin into DC Bead more quickly and to inject high amounts of anthracycline agent directly into the tumor warranting a sustained high-dose delivery over time while embolizing the tumor feeding arteries. The rapid incorporation of epirubicin was supposed to be attained by increased incidence of mechanical collision among chemical groups of substances. Compared to letting stand in room temperature, ultrasonic vibration or heating of solution were not effective in shortening the loading time of epirubicin.

We serially examined morphological changes of DC Bead after agitation with Vortex mixing, together with ultrasonic vibration and heating, comparing to mere leaving at room temperature. Microscopic examination disclosed Vortex mixing did not affect any morphological changes and characteristics of red-colored DC Bead, and the procedure was considered safe as an embolic agent. The rationale for the loading of the drug is the formation of an ionic attraction between the negatively charged sulfonate groups in DC Bead and the positively charged amino function on epirubicin. There is an initial diffusion of drug into the polymer matrix of DC Bead, and then the ionic binding occurs. It is believed that the diffusion of the drug into the beads is the rate controlling step for loading of DC Bead as smaller beads load much more rapidly due to their increased surface area. Since 30 seconds of mechanical agitation is not likely to induce ionic characteristics and ionic activity of sulfonic groups of the microspheres, binding of epirubicin to DC Bead seemed to be obtained successfully.

Release of the epirubicin is not considered to occur in the absence of an external impetus, and epirubicin remains within the bead for protracted periods of time in the absence of competing ions. To elute the drug, in vitro, a high ion concentration is required in solution to displace the epirubicin from the beads. Although we did not perform an experiment of the rapidity and rates of drug-release property from the agitated beads, the drug is considered to be released exclusively through ion exchange with sodium also from agitated beads.

In conclusion, DC Bead showed strong affinity for epirubicin, which had a similar structure to that of doxorubicin. More than 90% of the epirubicin dose (50 mg/mL) was incorporated into DC Bead at 5 minutes after reconstitution when the solution was agitated for 30 seconds with a Vortex mixer. This method may be useful in clinical settings requiring rapid performance of TACE and may also contribute to saving time during routine hospital procedures.

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