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In Vivo Drug Delivery Performance of Lipiodol-Based Emulsion or Drug-Eluting Beads in Patients with Hepatocellular Carcinoma

Elsa Lilienberg,[†] Ilse R. Dubbelboer,[†] Amar Karalli,[‡,§] Rimma Axelsson,[‡,§] Torkel B. Brismar,[‡,§] Charlotte Ebeling Barbier,[∥] Agneta Norén,[⊥] Frans Duraj,[⊥] Mikael Hedeland,[#] Ulf Bondesson,[#] Erik Sjögren,[†] Per Stål,[▽,○] Rickard Nyman,[∥] and Hans Lennernäs*[,†]

[†]Department of Pharmacy, Uppsala University, Box 580, 751 23 Uppsala, Sweden

[‡]Department of Radiology, Karolinska University Hospital in Huddinge, Stockholm, Sweden

[§]Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden

[∥]Department of Radiology, Uppsala University Hospital, Uppsala University, 751 85 Uppsala, Sweden

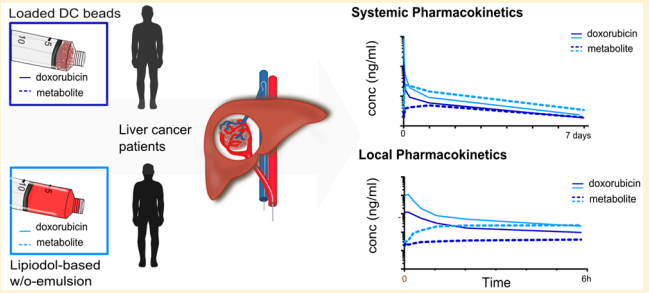
[⊥]Department of Surgical Sciences, Uppsala University Hospital, Uppsala University, 751 85 Uppsala, Sweden

[#]Department of Chemistry, Environment and Feed Hygiene, National Veterinary Institute (SVA), 751 89 Uppsala, Sweden

[▽]Unit of Gastroenterology, Deptartment of Internal Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden

[○]Department of Digestive Diseases, Karolinska University Hospital in Huddinge, Stockholm, Sweden

ABSTRACT: Doxorubicin (DOX) delivered in a lipiodol-based emulsion (LIPDOX) or in drug-eluting beads (DEBDOX) is used as palliative treatment in patients with intermediate-stage hepatocellular carcinoma (HCC). The primary objective of this study was to evaluate the *in vivo* delivery performance of DOX from LIPDOX or DEBDOX in HCC patients using the local and systemic pharmacokinetics of DOX and its main metabolite doxorubicinol (DOXol). Urinary excretion of DOX and DOXol and their short-term safety and antitumor effects were also evaluated. In this open, prospective, nonrandomized multicenter study, LIPDOX (n = 13) or DEBDOX (n = 12) were injected into the feeding arteries of the tumor. Local (vena cava/hepatic vein orifice) and systemic (peripheral vein) plasma concentrations of DOX and DOXol were determined in samples obtained up to 6 h and 7 days after treatment. Tumor response was assessed using computed tomography or magnetic resonance imaging. The C_{max} and $AUC_{0-24\ h}$ for DOX were 5.6-fold and 2.4-fold higher in LIPDOX vs DEBDOX recipients, respectively ($p < 0.001$). After 6 h, the respective mean proportions of the dose remaining in the liver or drug-delivery system (DDS) were 49% for LIPDOX and 88% for DEBDOX. LIPDOX releases DOX faster than DEBDOX in HCC patients and provides more extensive local and systemic exposure (AUC) to DOX and DOXol initially (0−7 days). DEBDOX formulation has a release and distribution of DOX that is more restricted and rate controlled than LIPDOX.



KEYWORDS: *doxorubicin, doxorubicinol, drug eluting beads, local delivery, local therapy, hepatocellular carcinoma, liver cancer, lipiodol, transarterial chemoembolization, transarterial infusion chemotherapy*

1. INTRODUCTION

Primary liver cancer was the sixth most common cancer form globally in 2012, with 782,000 new cases.[1] The incidence of liver cancer is expected to increase by 23% in the developed world and 37% globally by 2025.[2] About 20% of all diagnosed hepatocellular carcinomas (HCCs) are intermediate-stage, where the tumor is unresectable but there is no evidence of vascular invasion or extrahepatic spread.[3] Patients with intermediate-stage HCC are offered palliative treatment administered in drug-delivery systems (DDSs) loaded with chemotherapeutic drug(s).[3] These DDSs are administered close to the tumor (locoregionally) via the hepatic artery and holds embolic properties in various degrees. The delivery technique is called transarterial chemoembolization (TACE).[4] As the hepatic artery contributes with only 20−25% of the total liver blood supply but supplies >90% of the tumor, it is hoped that intra-arterial delivery and the induced ischemia will be disease-selective, will spare the surrounding liver, and will reduce other systemic, off-target effects.[5,6]

The multitarget, broad-spectrum, potent, cytostatic drug doxorubicin (DOX) is commonly delivered by DDSs used in intra-arterial delivery, either as a doxorubicin-in-lipiodol emulsion (LIPDOX) or in doxorubicin-eluting beads (DEB-

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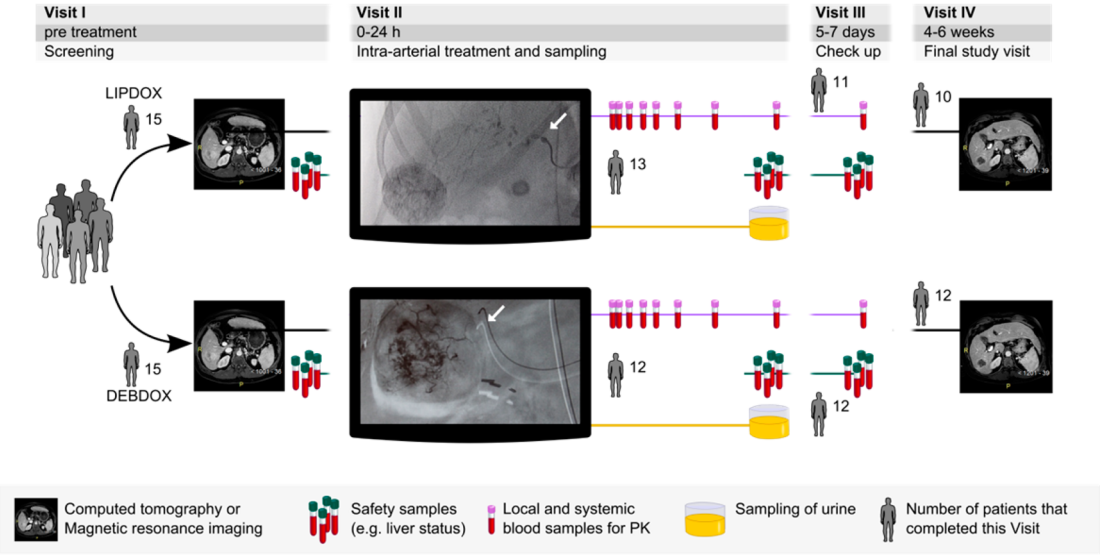

Figure 1. Overall design of this clinical study. Each patient was scheduled for four visits. Visit I) Screening, blood sampling for safety and computed tomography (CT) or magnetic resonance (MR) imaging session before treatment. Visit II) Intra-arterial procedure (time = 0) where the patient received a dose of either LIPDOX (n = 13) without additional embolic materials or DEBDOX (n = 12) to a previously untreated area of the liver. Before the dose was given, a catheter was inserted into the vena cava at orifice to the hepatic vein (local sampling site) and the femoral vein (systemic sampling site). The LIPDOX catheter was guided to the lobe (arrow) to enable treatment of possible tumor satellites. During injection of LIPDOX, lipiodol was monitored as small pulsating droplets approaching the tumor, followed by an accumulation in tumor. These droplets caused partial embolization. During injection of DEBDOX, the catheter was placed much closer to the target tumor (arrow). A contrast agent was added during the infusion to ensure that complete embolization was accomplished. Blood was collected at specific time points (t = 0−24 h). The extra catheter for local sampling was removed at 6 h. Urine was quantitatively collected between 0 and 24 h. During visits III and IV, final systemic blood samples and CT or MR images were taken, respectively. Of 13 included patients, 11 performed all visits in the LIPDOX group and 12 of 12 patients in the DCBDOX treatment group. Each patient participated in the study for up to 6 weeks after the intra-arterial treatment. DEBDOX = doxorubicin-eluting beads; LIPDOX = doxorubicin-in-lipiodol emulsion; PK = pharmacokinetics.

DOX).[7−9] LIPDOX can either be infused alone or the infusion can be followed by additional embolic materials, such as drug-free gelatin sponges or microparticles, administered to further reduce the local blood flow. DEBDOX causes complete embolization by itself.[4] LIPDOX has been used in the treatment of HCC since the 1980s,[10] but DEBDOX was only approved in the EU as a medical device in 2007.[11,12] There are no reported differences in overall survival between these two treatments, and both therapies have a one- and two year survival of about 70% and 45%, respectively.[13] The similar survival outcomes between LIPDOX with additional embolic materials and DEBDOX were supported in a meta-analysis with strict inclusion criteria, although it was found that the objective tumor response rate was higher with DEBDOX.[14]

The excipient Lipiodol in LIPDOX is a poppy seed oil derivate (480 mg iodine/mL) and shortly after infusion, lipiodol is available in the vascular lumen, in the endothelial lining, and inside the tumor cells (apparent as a result of endocytosis).[15,16] The excipient lipiodol generates transient embolization of the sinusoidal vessels and subsequent portal venules in animal studies.[17,18] Lipiodol has been reported to accumulate in the tumor for a longer period (days/months) as a result of the dynamic pathophysiological changes in the vascular tumor environment that enhance the permeability and retention of macromolecules.[15,19] Using image guidance, LIPDOX can be infused from a segmental, lobar, or superselective placement.

The DDS DEBDOX used in this study consist of biocompatible, nondegradable microparticles (DC Bead), which are available in four sizes in the range 70−700 μm.[20] The beads are poly vinyl alcohol (PVA)-based hydrogels with incorporated chains of acrylamido-2-methylpropanesulfonate sodium salt.[20] Positively charged DOX binds to the negatively charged sulfonate groups in the beads. Loading and release of DOX requires counterions, as both mass processes are driven by ion-exchange and diffusion.[20,21] More specifically, novel data suggest that the release rate is controlled by the outer film of the bead, as well as influenced by DOX-DOX aggregates, DOX−PVA interactions, and the equilibrium between protonated and deprotonated DOX.[22] Using image guidance, DEBDOX are administered in the immediate vicinity of the tumor (superselective).[20]

Early clinical pharmacokinetic (PK) data for DOX released from these DDSs in patients showed slow release from DEBDOX with subsequent low systemic plasma exposure (area under the concentration−time curve; $AUC_{0-7\ days}$).[11,12,23] When about the same intrahepatic doses of DOX (100−150 mg) are administered, DEBDOX generates fewer systemic DOX-related side effects than LIPDOX.[24] A thorough comparison of DOX PK from these DDSs undertaken in healthy pigs indicated that the release of LIPDOX is faster and more extensive *in vivo* than that of DEBDOX, resulting in higher systemic plasma exposure.[25]

The main objective of this short-term study was to compare the *in vivo* release of DOX from two clinical regimens using intra-arterial administration of the most stable LIPDOX emulsion or DEBDOX in HCC patients. This was assessed by thoroughly investigating the local and systemic PK of DOX and its primary metabolite doxorubicinol (DOXol). The vena cava orifice from the hepatic vein was used as the local sampling site in an attempt to improve understanding of the local release and disposition of the drug close to the tumor site and in the

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rest of the liver. To our knowledge such thorough PK comparison with these formulations has not previously been performed in HCC patients. The secondary objectives were to examine urinary excretion of DOX and DOXol and to relate the PK to short-term toxicity and antitumor effects.

2. PATIENTS AND METHODS

2.1. Patients and Treatment. *2.1.1. Study Design.* This study was designed as an open, prospective, nonrandomized, multicenter study (Figure 1). The included patients were divided into two study arms based on the standard treatment for these patients at the hospital to which they were admitted: The patients admitted to Uppsala University Hospital were assigned LIPDOX treatment without infusion of any additional embolizing materials, and the patients admitted to Karolinska University Hospital were assigned DEBDOX treatment. Each patient should attend four visits (I−IV) over a period of up to 6 weeks after one course of treatment; At visit I, the patients were screened with blood sampling and baseline computed tomography (CT) scan or magnetic resonance (MR) image were taken. At visit II, the intra-arterial treatment with the DDS was performed and blood samples were taken for PK and safety monitoring. Urine was collected up to 24 h after treatment. At visit III, additional blood samples for PK and safety were taken and at visit IV a CT or MR imaging follow-up took place.

The protocol was approved by the regional ethical review board in Uppsala (Dnr 2013/227) and registered in the European Clinical Trials Database (EudraCTnr 2013-001244-56). All enrolled patients provided written informed consent.

2.1.2. Inclusion and Exclusion Criteria. To be included in the study, each patient (>18 years of age) had to be diagnosed with intermediate-stage HCC (BCLC B), defined as a Child−Pugh score of A or B (max B7) and ECOG performance status of 0−2. The life-expectancy of the patient should be ≥3 months in absence of medical treatments and the following lab data criteria should be met: creatinine ≤ 115 μmol/L, bilirubin ≤ 35 μmol/L, albumin ≥ 28 g/L, leukocytes ≥ 1.5×10^9/L, INR ≤ 1.7. Lab values were considered to be normal when albumin ≥ 36−45 g/L; bilirubin ≤ 26 μmol/L; creatinine ≤ 100 μmol/L (men), ≤ 90 μmol/L (women); INR ≤ 1.2; leukocytes 0.7−3.9 × 10^9/L.

Patients were excluded if any of the following exclusion criteria were met; infiltrative HCC, undefined liver tumor(s), which were immeasurable or not assessable, portal vein thrombosis with the exception of thrombosis of a segment branch of the portal vein, extrahepatic cancer involvement, contraindications to arteriography or to DOX and other anthracyclines, and ascites grades 2 or 3. Patients were also excluded if they were pregnant, had any systemic or local infections (except for HIV responsive to therapy, hepatitis B virus, or hepatitis C virus), or if they had received any prior treatment with DOX during the last three months or have had any prior TACE treatment.

2.1.3. Preparations of Drug-Delivery Systems (DDSs) Used in the Intra-arterial Procedure. The aqueous and lipid parts of LIPDOX were prepared by the hospital pharmacy (Apoteket Farmaci) at Uppsala University Hospital in two separate syringes joint by a connector. The aqueous DOX solution consisted of 2.56 mL of iohexol (Omnipaque 300 mg I/mL; GE Healthcare, Stockholm, Sweden), 0.44 mL of sterile water, and 50 mg of DOX hydrochloride (Adriamycin; Pfizer Inc., New York, NY). Iohexol is a contrast agent used as a densifier to stabilize the emulsion.[17] The other syringe contained 10 mL of lipiodol (Lipiodol Ultra Fluide, Guerbet, Aulnay-sous-Bois, France). According to standardized procedure, the syringe contents were mixed manually by syringe-pumping approximately 10−15 times just prior to administration to make a LIPDOX (o/w) emulsion (3.3:1).

The DC Bead beads (Biocompatibles, Surrey, UK) were used in the size ranges 70−150, 100−300, 300−500, and 500−700 μm. DOX was loaded into the microparticulate hydrogel beads by APL, Stockholm, Sweden. The beads were loaded with 37.5 mL of DOX hydrochloride aqueous solution (2 mg/mL, Teva Parenteral Medicines Inc., Irvine, CA) per 2.0 mL of beads according to the manufacturer's protocol.[26] The prepared syringes contained approximately 5 mL of DEBDOX. At the Karolinska University Hospital, DEBDOX was dispersed (1:1) in the contrast agent iodixanol (Visipaque 270 mg I/ml; GE Healthcare, Oslo, Norway) for visualization during treatment.[27,28] Just prior to administration, smaller administration portions containing 2.0 mL of the DEBDOX-contrast solution, 2.0 mL of additional iodixanol, and 2.0 mL of physiological saline were prepared.

2.1.4. Transarterial Procedures and Intended Doses. The transarterial procedures were performed under fluoroscopic guidance by an experienced interventional radiologist. The common femoral artery was punctured and a 5 Fr introducer was placed therein. Through this introducer, selective catheterization of the celiac trunk and the hepatic artery was carried out with either a 4 Fr catheter or an additional microcatheter. The femoral vein was punctured and a 7 Fr introducer followed by a 5 Fr catheter that was placed in the vena cava at orifice from the hepatic vein (VC/VH) for local sampling. The introducer in the femoral vein enabled access to the iliac vein for systemic sampling during the 6 h after treatment, while the VC/VH catheter was still in position. LIPDOX and DEBDOX were administered slowly by hand until complete embolization was reached or until the intended maximum dose was infused. The placement of catheter (lobar/segmental/superselective) and the dose given were chosen based on the standard treatment at each clinic. LIPDOX (50 mg DOX) was administered lobar and DEBDOX (150 mg DOX) was administered superselective.[28,29] The individual dose of each DDS was monitored. The choice of DEBDOX bead size was based on the size of the vessels for the tumor in each patient.[28] If complete stasis was not met with the maximum dose of DEBDOX, embolization was completed with additional unloaded beads. No additional embolization materials were used in the LIPDOX treatment.

2.2. Doxorubicin and Doxorubicinol Pharmacokinetics. *2.2.1. Blood and Urine Sampling.* In all patients, a pretreatment sample was collected from a peripheral vein. If the administration time was long, extra samples were taken every 10 min (LIPDOX) or 15 min (DEBDOX) after infusion start until the end of infusion. After the infusion, local (VC/VH) and systemic (peripheral vein) samples were collected at 0, 5, 15, and 30 min and 1, 2, and 6 h. After 6 h, the VC/VH catheter was removed. Additional systemic samples were collected at 24 h and 5−7 days after administration. Blood samples were collected in EDTA-containing vacutainers (4 mL, BD Biosciences, Franklin Lakes, NJ, USA) and centrifuged (10 min, 18 °C, 3600*g*). The plasma was thereafter transferred to dark polypropylene tubes before storage at −20 °C until analysis. Urine was collected from the end of the treatment for 24 h. The urine was weighed, and aliquots were collected in dark polypropylene tubes and stored at −20 °C until analysis.

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All samples were protected from light because of DOX photosensitivity.

2.2.2. Drug Analysis. A Waters Acquity UPLC system coupled to a Quattro Ultima Pt tandem quadrupole mass spectrometer (UPLC−MS/MS, Waters Corporation, Milford, MA) was used for the drug analysis. DOX hydrochloride and DOXol hydrochloride were obtained from LC Laboratories (Woburn, MA) and Toronto Research Chemicals (North York, ON, Canada), respectively. The internal standards [^{13}C, ^{2}H$_3$]-DOX trifluoroacetate and [^{13}C, ^{2}H$_3$]-DOXol trifluoroacetate were purchased from Alsachim (Illkirch-Graffenstaden, France). All other chemicals were of analytical grade or higher. The UPLC−MS/MS methods used to quantify DOX and DOXol in human plasma and urine were the same as those used for porcine plasma and urine as previously described by our group.[25,30] The sample preparation for plasma was adopted from Dubbelboer et al.[30] For both DOX and DOXol, the lower limit of quantification (LLOQ) was 0.5 ng/mL in plasma and 2.5 ng/mL in urine. The precision, i.e., the relative standard deviation (RSD), for the results of quality control samples in plasma was 2.3−5.6% (DOX) and 3.0−6.5% (DOXol). In urine, the RSD was 2.5−4.8% for DOX and 1.6−3.3% for DOXol.

2.2.3. Pharmacokinetic (PK) Data Analysis. The plasma concentration−time profiles for DOX and DOXol were analyzed by noncompartmental analysis using Phoenix WinNonLin 6.3. The AUC was calculated from the start of the drug infusion, using linear and logarithmic trapezoidal methods for the ascending and descending parts of the curve, respectively. The terminal rate constants, used to calculate terminal half-life from the two last observed time points (between 6 and 24 h [λ_{24h}] or between 24 h and 5−7 days [$\lambda_{5-7days}$]), were dependent on sample availability for each patient. Plasma sample concentrations below LLOQ were excluded. Plasma AUC was reported as several partial AUCs (AUC_{last}, AUC_{0-6h}, AUC_{0-24h}, AUC_{0-5d}, AUC_{0-7d}) whereof only patients with samples within the specific time frame were included in the parameter.

Since the pharmacokinetics of DOX are linear,[31] the *in vivo* PK parameters (AUC and maximum concentration, C_{max}) were dose-normalized to enable comparison of the parameters for LIPDOX and DEBDOX according to eq 1:

$$\text{dose-normalized parameter} = \left(\frac{\text{parameter}_{\text{individual}}}{\text{dose}_{\text{individual}}(\text{mg})}\right)\cdot 100\ \text{mg} \quad (1)$$

where 100 mg was used for two reasons; to maintain the dose unit and because it is the average intended doses of the DDSs in this study.

In this article, the bioavailability (F) reflects the fraction of the DOX dose released from LIPDOX or DEBDOX intrahepatically and subsequently reaches the systemic circulation. An intravenous (i.v.) injection of solution was applied as a reference as it has a complete bioavailability. Here, the bioavailability of DOX was estimated by deconvolution using WinNonLin software.[32] Reference i.v. data for DOX in HCC patients were taken from the literature.[23,32] The reference data[23] was best described by a three-compartment model from which the weighting parameters for the deconvolution were collected. The fraction of the DOX dose that reached the systemic circulation after 6 or 24 h is reflected in the systemic bioavailability, and the fraction of the dose reaching the VC/VH measurement site after 6 h was defined as the local bioavailability (F_{local}). As the administration site is within the liver and the local sampling site is directly after the liver, the remaining dose fraction (RD, %) in the liver after 6 h is the dose fraction extracted by the liver or left in the DDS:

$$\text{RD}(\%) = 100 - F_{\text{local,6h}} \quad (2)$$

2.3. Safety and Tumor Response. The secondary objective of this study was to assess the short-term toxicity and antitumor effect of LIPDOX and DEBDOX. The incidence of adverse events were monitored up to 4−6 weeks after treatment, and its severity was registered. Blood samples were collected on three occasions: prior to treatment, and 24 h and 5−7 days post-treatment. Liver function [aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP), prothrombin-international normalized ratio (INR), and bilirubin], kidney function (creatinine and urea), blood count (hemoglobin, thrombocytes, leukocytes and albumin), electrolytes (sodium and potassium), and inflammatory response [C-reactive protein (CRP)] were monitored. The analyses of these samples were performed at the clinical chemistry lab at each hospital.

The antitumor effects were investigated using dynamic contrast-enhanced MR imaging or 4-phase multidetector CT scans before and 4−6 weeks after LIPDOX or DEBDOX treatment. In order to reduce bias caused by the contrast effects of lipiodol, only MR imaging was used for the LIPDOX group. Scans from both study centers were reviewed independently by four experienced radiologists in a nonblinded fashion. Tumor response was evaluated using the modified-response evaluation criteria in solid tumors (mRECIST) criteria.[33]

2.4. Statistical Analysis. Student's unpaired t test was performed to compare PK parameters between the DDSs. Safety parameters such as ASAT and ALAT concentrations were compared with Student's unpaired t test. The change in any safety parameter between visit II (24 h post-treatment) and baseline was compared with the change between visit III (5−7 days post-treatment) and baseline. The Fisher's exact test was performed to compare tumor response between LIPDOX and DEBDOX. Statistical significance was set as $p < 0.05$. Bonferroni correction was used to adjust for multiple comparisons. Continuous variables were expressed as mean ± standard deviation (SD) unless stated otherwise.

3. RESULTS

3.1. Patients and Treatment. From January 2014 to March 2016, 30 HCC-patients were included in this clinical multicenter study (Figure 1). Of those 30 patients, five patients were excluded prior to treatment. This resulted in study continuation with treatment and sample collection (Visit II) for 13 patients in the LIPDOX group and 12 patients in the DEBDOX group. Two patients in the LIPDOX group were withdrawn from the study after visit II because of liver failure, whereof one resulted in death. One patient in the DEBDOX group was excluded from the PK analysis (but not the tumor response or safety analyses) because of hemolysis in the initial blood samples. A total of 21 patients completed the short-term effect evaluation in visit IV (Figure 1). The baseline characteristics of the 25 included patients were similar in both treatment groups and are presented in Table 1. However, the tumors to be treated in the LIPDOX group were larger ($p <$

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0.05) at screening than those in the DEBDOX group. Most of the patients (80%) had multifocal liver tumors.

Table 1. Demographic Data for the 25 Patients Included in This Clinical Study, between January 2014 and March 2016

characteristics	LIPDOX	DEBDOX
number of patients included	13	12
mean age[a] (years)	69 (55−83)	73 (53−85)
gender (male/female)	11/2	9/3
weight (kg)	83 (56−99)	81 (61−110)
etiology of HCC[b] (HCV or HBV/alcohol/other)	3/1/9	5/1/6
Child−Pugh (A/B/na)	10/3	10/2
ECOG performance status (0/1/2)	10/3/0	5/5/2
mean tumor size[a,c] (mm)	62 (30−130)	36 (6−65)[e]
type of tumor (unifocal/multifocal)	4/9	1/11
Baseline Lab Data[d]		
albumin (g/L)	33 ± 9.6	32 ± 3.9
bilirubin (μmol/L)	15 ± 5.1	15 ± 13
creatinine (μmol/L)	74 ± 21	72 ± 22
INR	1.2 ± 1.8	1.2 ± 0.2
leukocytes (10^9/L)	5.7 ± 1.7	6.7 ± 2.5

[a]Presented as mean (range). [b]HCV, hepatitis C virus; HBV, hepatitis B virus. [c]Tumor size was measured as the maximum diameter of the tumor to be treated. [d]Inclusion criteria were albumin ≥ 28 g/L; bilirubin ≤ 35 μmol/L; creatinine ≤ 115 μmol/L; INR ≤ 1.7; leukocytes ≥ 1.5 × 10^9/L. [e]*p < 0.05. na = not available.

3.2. Doxorubicin and Doxorubicinol Pharmacokinetics. The plasma concentration−time curves for DOX and DOXol following administration of LIPDOX or DEBDOX are displayed in Figure 2a,b. In the LIPDOX group, all 13 patients received a dose of 50 mg of DOX, and the PK parameters for DOX and DOXol are presented in Table 2. The mean infusion time for LIPDOX was 10 (range 2.0−50) min. The last plasma sample (i.e., at 5−7 days) was below the LLOQ for 27% of the patients that were investigated in the LIPDOX group. The terminal half-life$_{5-7d}$ of DOXol was similar to that of DOX, which was 54 ± 8 h (Table 2). In the DEBDOX group, the mean administered dose was 83 mg (range 22.5−150 mg). The resulting PK parameters for DOX and DOXol are presented in Table 2. The DEBDOX dose resulted in complete embolization in 60% of the patients. The remaining five patients received additional unloaded beads to complete embolization. The DEBDOX bead sizes given in different combinations to the patients were 70−150 μm (six patients), 100−300 μm (ten patients), and 300−500 μm (four patients). The mean infusion time was 13 (range 3.0−34) min. The 5- to 7-day plasma sample was below the LLOQ for 11% of the patients in the DEBDOX group. The terminal half-life$_{5-7d}$ was slightly longer for DOXol than for DOX (120 ± 53 h and 99 ± 99 h, respectively). For both DDSs, C_{max} of DOX occurred about 5 min after the infusion ended.

The plasma concentration−time profiles and PK parameters for LIPDOX and DEBDOX were compared after normalizing

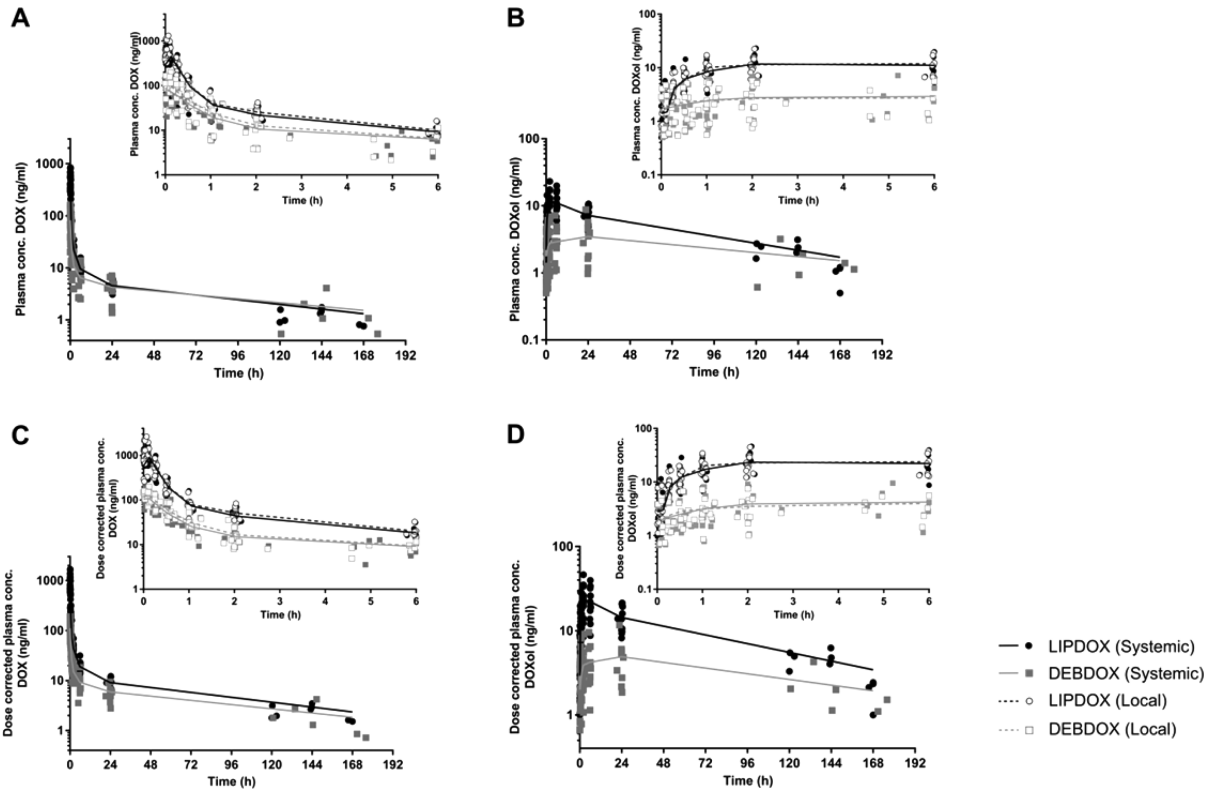

Figure 2. Plasma concentration−time profiles for doxorubicin (DOX; A and C) and doxorubicinol (DOXol; B and D). Panels A and B show the actual concentrations of DOX and DOXol in the plasma samples. Panels C and D show the dose-normalized plasma concentration−time profiles. Infusion of doxorubicin-lipiodol emulsion (LIPDOX) or doxorubicin-eluting beads (DEBDOX) was initiated at time 0. Samples were taken locally up to 6 h in the vena cava orifice from the hepatic vein (local) or systemically in the femoral vein up to 168 h (7 days) to reflect the circulation (systemic) after hepatic injection of LIPDOX or DEBDOX using intra-arterial procedures. The insets show the systemic and local plasma concentration−time profiles during 6 h. Lines represent the means for the study arm, setting the last collection point to 168 h for all patients, although the last point could have been between 120 and 168 h. Symbols represent the individual data and are plotted against the time of collection.

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Table 2. Systemic Plasma Pharmacokinetic (PK) Parameters for Doxorubicin and Doxorubicinol Following Transarterial Delivery of Doxorubicin-Lipiodol Emulsion (LIPDOX; Dose 50 mg) or Doxorubicin-Eluting Beads (DEBDOX; Dose 83 mg, Range 22.5−150 mg) to All Patients[a]

	doxorubicin		doxorubicinol	
PK parameters	LIPDOX	DEBDOX	LIPDOX	DEBDOX
C_{max} (ng/mL)	480 ± 240 (13)	85 ± 46 (11) ***	12 ± 5.2 (13)	3.8 ± 2.4 (11) **
AUC_{0-24h} (h·ng/mL)	340 ± 170 (13)	140 ± 120 (11)	200 ± 110 (12)	51 ± 53 (10) *
t_{max} (min)	12 ± 5.1 (13)	16 ± 9.3 (11)	160 ± 120 (13)	1700 ± 2400 (11)
half-$life_{24h}$ (h)	16 ± 0.7 (4)	42 ± 31 (3)	17 (1)	94 ± 7.3 (2)
half-$life_{5-7days}$ (h)	54 ± 8 (8)	99 ± 99 (7)	58 ± 12 (11)	120 ± 53 (6) *

[a]All data shown as means ± SD(n), where n is the number of patients. Significant differences compared to the same parameter for LIPDOX are indicated by *p < 0.05; **p < 0.01; ***p < 0.001. AUC = area under the concentration−time curve; C_{max} = maximum concentration; NA = not available; t_{max} = time to C_{max}.

Table 3. Dose-Normalized Plasma and Urine Pharmacokinetic (PK) Parameters for Doxorubicin and Doxorubicinol Following Transarterial Delivery by Doxorubicin-Lipiodol Emulsion (LIPDOX) or Doxorubicin-Eluting Beads (DEBDOX)[a,b]

		doxorubicin		doxorubicinol	
		LIPDOX	DEBDOX	LIPDOX	DEBDOX
Plasma					
systemic	C_{max} (ng/mL)	960 ± 470 (13)	110 ± 41 (11) ***	25 ± 10 (13)	5.1 ± 2.7 (11) ***
	AUC_{0-6h} (h·ng/mL)	540 ± 170 (13)	140 ± 53 (11) ****	120 ± 48 (13)	19 ± 12 (11) ****
	AUC_{0-24h} (h·ng/mL)	810 ± 220 (12)	270 ± 110 (10) ****	460 ± 150 (12)	100 ± 59 (10) ****
	AUC_{0-5d} (h·ng/mL)	1400 ± 380 (8)	670 ± 230 (7) *	1300 ± 400 (11)	450 ± 230 (7) **
	AUC_{0-7d} (h·ng/mL)	1200 ± 6.1 (2)	720 ± 300 (3)	1100 ± 220 (5)	630 ± 330 (3)
local	C_{max} (ng/mL)	1200 ± 670 (13)	130 ± 44 (11) **	26 ± 10 (13)	4.2 ± 1.9 (11) ****
	AUC_{0-6h} (h·ng/mL)	630 ± 190 (13)	140 ± 61 (11) ****	130 ± 47 (13)	15 ± 7.4 (11) ****
systemic to local ratio	C_{max}	0.86 ± 0.23 (13)	0.94 ± 0.27 (11)	0.95 ± 0.17 (13)	1.2 ± 0.27 (11)
	AUC_{last}	0.88 ± 0.16 (13)	0.96 ± 0.22 (11)	0.97 ± 0.14 (13)	1.5 ± 0.92 (10)
Urine	f_e (%)	2.8 ± 2 (10)	0.61 ± 0.38 (8)	0.8 ± 0.63 (10)	0.11 ± 0.12 (8)

		systemic		local	
		LIPDOX	DEBDOX	LIPDOX	DEBDOX
DOXol to DOX ratio					
Bioavailability[c]	AUC_{las}	0.82 ± 0.32 (13)	0.57 ± 0.27 (11)	0.21 ± 0.089 (13)	0.1 ± 0.026 (11) *
	F_{6h} (%)	51 ± 17 (13)	12 ± 4.6 (11) ****	60 ± 21 (13)	14 ± 5.6 (11) ****
	F_{24h} (%)	51 ± 17 (13)	15 ± 6.1 (11) ****	NA	NA
	RD (%)[d]	49 ± 17 (13)	88 ± 4.6 (11) ****	NA	NA

[a]All data shown as means ± SD(n), where n is the number of patients. [b]Doses were normalized by dividing the parameter result by the individual dose and multiplying it by 100 mg (eq 1). [c]These data were derived from deconvolution using historical i.v. data.[22] [d]This parameter was based on individual F and was calculated for each patient. Significant differences compared to the same parameter for LIPDOX are indicated by *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. AUC = area under the concentration−time curve; C_{max} = maximum concentration; F = bioavailability; f_e = dose fraction excreted to urine; RD = remaining dose.

the dose according to eq 1 (Table 3, Figure 2c−d). Plasma C_{max} was 8-fold higher for LIPDOX than for DEBDOX (960 vs 110 ng/mL, p < 0.001) and mean AUC_{0-24h} values were 4-fold higher (540 vs 140 h·ng/mL, p < 0.0001). AUC_{0-5d} for both DOX and DOXol was significantly higher with LIPDOX than with DEBDOX, and AUC_{0-7d} was higher as well, although the difference was not significant. At the local sampling site (VC/VH), plasma exposure to DOX and DOXol was higher with LIPDOX than with DEBDOX (630 vs 140 h·ng/mL and 130 vs 15 h·ng/mL, respectively, p < 0.0001) (Table 3). The individual ratios of local to systemic plasma DOX concentrations directly after administration (time 0) were 1.57 ± 0.63 and 1.21 ± 0.14 in the LIPDOX and DEBDOX groups, respectively. This difference lessened with time, to stabilize at 15−30 min around 1.1−1.2 in both groups (Figure 2). The systemic bioavailability of DOX after 24 h, F_{24h}, was 3.3-fold higher with LIPDOX compared to with DEBDOX (p < 0.0001). The local bioavailability after 6 h, F_{6h}, was 4.3 times higher with LIPDOX than with DEBDOX (p < 0.0001). The RD of DOX left in the liver/formulation after 6 h was about 1.8 times higher for DEBDOX than for LIPDOX (p < 0.001).

3.3. Safety and Tumor Response. During the study period (visits II−IV) the following adverse events were reported. In the LIPDOX group, clinically relevant postembolization syndrome occurred in four (31%) patients. One patient (8%) experienced liver failure and one suffered from circulatory arrest after LIPDOX treatment, leading to patient withdrawal from the study. Other reported adverse events in the LIPDOX group that were probably unrelated to the treatment drug were: rectal bleeding (n = 1, 8%), bleeding from the catheter placement site (n = 1, 8%), confusion (n = 1, 8%), and headache (n = 1, 8%). In the DEBDOX group, four (33%) patients developed postembolization syndrome, one patient (8%) had an infection in the necrotic part of the liver after treatment, two patients (17%) had back pain, and one patient (8%) had a urinary tract infection. No patients developed liver failure in the DEBDOX group. Common systemic DOX-related adverse events such as alopecia, myelosuppression, and dyspnea

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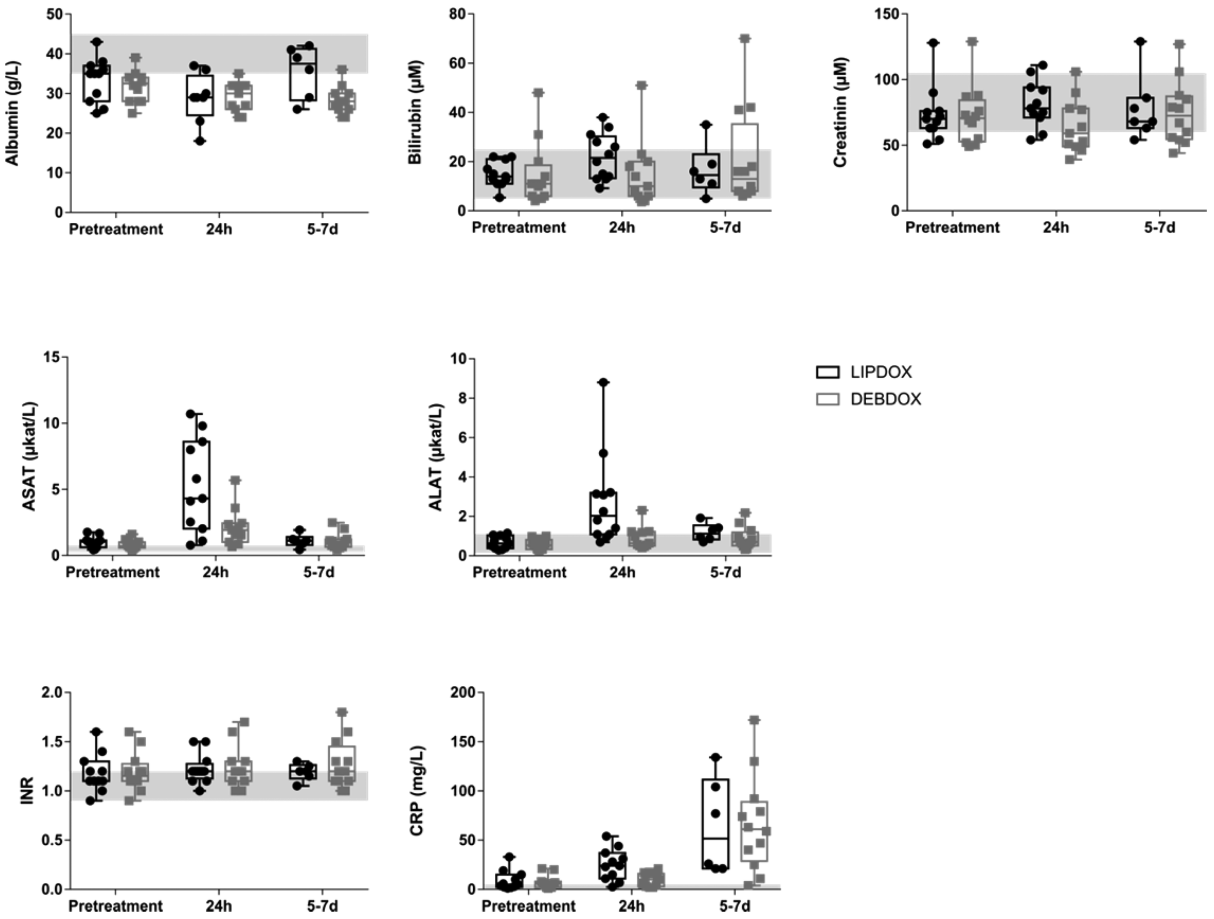

Figure 3. Safety parameters, monitored from visit I (before treatment), through visits II (24 h after treatment) and III (5−7 days after treatment), are presented as individual data. The monitored parameters included blood concentrations of albumin, bilirubin and creatinine, aspartate amino transferase (ASAT), alanine amino transferase (ALAT), prothrombin-international normalized ratio (INR), and C-reactive protein (CRP). Each symbol represents an individual patient. The gray area in each plot shows the reference values for healthy persons.

did not occur in any of the patients receiving LIPDOX or DEBDOX during the study period.

There were no statistical differences between the treatment groups or between visits within each treatment for any of the monitored safety parameters during the study period. The values of some of these parameters are presented in Figure 3. In some patients, the ASAT and ALAT concentrations were more elevated 24 h post-treatment in the LIPDOX group compared to the DEBDOX group. However, after 5−7 days the individual ASAT and ALAT concentrations had declined and were approaching its baseline again (Figure 3). In both treatment groups, CRP increased with time after treatment, indicating that an inflammatory response was induced by the treatment. CRP did not differ between the groups.

The mRECIST tumor responses are presented in Table 4, with no significant differences between the groups. The overall response (complete and partial response combined) was 67% (n = 6/9) for LIPDOX and 91% (n = 10/11) for DEBDOX, and disease control (complete response, partial response, and stable disease combined) was 100% (n = 9/9) for LIPDOX and 91% (n = 10/11) for DEBDOX. Representative MR images for LIPDOX and DEBDOX are presented in Figure 4.

Table 4. Treatment Responses According to mRECIST[a]

mRECIST	LIPDOX	DEBDOX
PD	0 (0%)	1 (9.1%)
SDi	3 (33.3%)	0 (0%)
PR	5 (55.6%)	8 (72.7%)
CR	1 (11.1%)	2 (18.2%)

[a]The Modified Response Evaluation Criteria in Solid Tumors include tumor size and the extent of necrotic changes to the tumor in the evaluation.[33] mRECIST classification was assessed by computed tomography or magnetic resonance imaging of the patients taken prior to and 4−6 weeks after transarterial treatment with doxorubicin-lipiodol emulsion (LIPDOX) or doxorubicin-eluting beads (DEBDOX). PD = progressive disease; SDi = stable disease; PR = partial response, and CR = complete response.

4. DISCUSSION

In this study, the drug delivery performance of LIPDOX and DEBDOX were compared in HCC patients, with the primary focus on the local (VC/VH) and systemic (peripheral vein) PK of DOX and DOXol. To our knowledge, this study is the first extensive PK comparison in patients of these two clinically used DDSs. LIPDOX administration resulted in a higher exposure to (AUC) and bioavailability of (F) DOX and DOXol than seen with DEBDOX, at both local and systemic sites. Conversion of DOX to DOXol was greater with LIPDOX than with DEBDOX, as reflected by several local PK parameters (local DOXol/DOX AUC ratio, C_{max} and AUCs for DOXol). These data are supportive of DOX being released faster and most likely exposing a larger internal liver area from LIPDOX than

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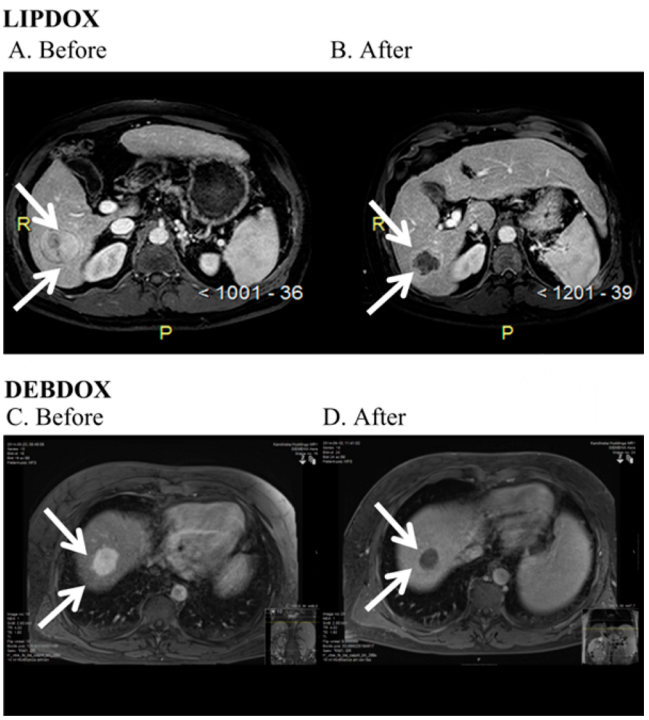

Figure 4. Representative contrast media-enhanced magnetic resonance (MR) scans from two patients before and one month after the first intra-arterial treatment with LIPDOX or DEBDOX. (A) A 48 × 43 mm hypervascular tumor before LIPDOX treatment (arrow); (B) 4−6 weeks after LIPDOX treatment, without additional embolic agents, showing a partial response with a central necrotic region in the now 35 × 24 mm tumor (arrow); (C) a 44 × 33 mm hypervascular tumor before DEBDOX treatment (arrow); and (D) 4−6 weeks after treatment with DEBDOX showing a complete response. The necrotic region is indicated by the arrow.

from DEBDOX, which had provided a more rapid enzymatic conversion to DOXol. Like many PK studies this study contains a small collection of patients, and therefore, effect and safety data should be carefully interpreted.

The C_{max} of DOX and the initial plasma exposure (AUC_{0-24h}) to DOX in the systemic compartment were 8.7- and 3.3-fold higher, respectively, after LIPDOX than after DEBDOX. This was comparable to previously reported data.[11] The time to reach peak concentrations of DOX from both DDSs (5 min after the end of the infusion) suggests an initial burst of DOX released into the systemic circulation from both DDSs followed by a rather rapid distribution phase (see Figure 2). The 2-fold longer half-lives$_{5-7d}$ for DOX when given as DEBDOX is in accordance with a slower release rate from DEBDOX than LIPDOX. It also shows that the release rate is slower than the elimination rate of DOX. The similar half-life$_{5-7d}$ for DOX and DOXol clearly indicates that the PK of DOXol is limited by its formation rate.

The mean dose-normalized AUC values for LIPDOX in this study were 3.3- to 4.9-fold lower than historic data presented by Johnson and colleagues,[23] although they used a similar dose of DOX (i.e., 60 mg/m^2). The difference in AUC may be explained by differences in the physical stability of the emulsions; Johnson and colleagues used a LIPDOX emulsion that contained a 2:5 volume ratio of lipiodol and aqueous DOX solution with normal saline, while we used a LIPDOX emulsion with a 3.3:1 volume ratio that has been reported to be more stable.[9,23,34,35] There is a direct relationship between physical *in vitro* stability and the release rate of drug from emulsion formulations *in vivo* (the higher stability, the lower release rate),[36] and therefore, the 3.3:1 LIPDOX emulsion may generate a lower AUC during the time given. However, emulsions are unstable formulations that can separate into two phases before the infusion and/or immediately in the blood vessel.[9] Indeed, also the most stable LIPDOX emulsions (3.3:1 volume ratio) have a low physical stability and starts to separate within 10 min *in vitro*.[34] A high stability of the emulsion may increase the amount of DOX transported together with lipiodol into the tumor environment via the enhanced permeability and retention mechanism that is specific to the tumor environment.[9,19] Hence, the difference in physical stability is a critical factor for the heterogeneous drug delivery observed with LIPDOX.

The DEBDOX beads used in this study (size range 70−700 μm) generated a mean dose-normalized C_{max} and AUC of DOX that was in agreement with data previously reported for DEBDOX$_{500-700\ \mu m}$.[12] However, Varela and co-workers reported a lower AUC_{0-7d} for DOX of 662.6 ± 417.6 min·ng/mL from DEBDOX$_{500-700\ \mu m}$ following a mean dose of 128 mg.[11] Varela's AUC value is 65 times lower than our dose-normalized mean (AUC_{0-7d} 43200 min·ng/mL), a disagreement that not only could be explained by the dose difference (100 vs 128 mg). It might be that an accidental error was made in the units for their AUC. Their LLOQ of 1 ng/mL and study period of 7 days (i.e., 10080 min) had to result in an AUC of >10080 min·ng/mL. If their result was actually in h·ng/mL but reported by mistake as min·ng/mL, then their AUC values would be very similar to ours (difference quota of 1.08). It is important to emphasize that DEBDOX drug delivery is heterogeneous, partly because of the differences in bead sizes use to individualize treatment, and partly because the primary treatment end point is to achieve vascular stasis rather than to give a specific dose of the drug. Higher doses and smaller beads are directly correlated with higher plasma exposure of DOX and DOXol.[9] A higher release rate of DOX has been observed in smaller beads *in vitro* and explained by the shorter diffusion path within the bead and the larger total surface area than a larger bead.[22,37] It has been reported that the *in vitro* release of DOX was not influenced by pH, which indicates that the pH in the tumor microenvironment would not much affect the drug release of DOX from the beads *in vivo*.[22] However, the influence of other tumor microenvironment factors, such as accumulation of various electrolytes and endogenous metabolites, on the *in vivo* release remains to be investigated.

The local DOXol-to-DOX AUC ratio was 2-fold higher following LIPDOX treatment than following DEBDOX treatment ($p < 0.001$; Table 3). This difference in ratio between the two formulations may be explained by a difference in their local distribution of DOX. Given the physical differences between the two formulations, it is comprehensible that emulsion based LIPDOX has a more extensive tissue distribution than the microparticle-based DEBDOX. One hypothesis is that the intracellular delivery of DOX from LIPDOX may be greater than that from DEBDOX as it is spread over a larger internal surface area. Higher intracellular concentrations of DOX would lead to more extensive formation of DOXol since its metabolism is mediated by intracellular cytosolic enzymes such as carbonyl reductases (mainly CBR1) and/or aldo-keto reductases (AKRs).[38,39] Indeed, higher intracellular availability of DOX has previously been reported *in vivo* following delivery with lipiodol-based

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emulsion and *in vitro* together with other lipids.[25,40−44] Several mechanisms such as lipid-enhanced membrane fluidity, inhibition of carrier-mediated efflux ABC transporters, increased aqueous solubility, and/or enzymatic hydrolysis have been proposed for an increased cellular permeability of DOX when it is coadministered with lipids.[40−43] However, we have recently shown *in vivo* that drug-free lipiodol does not inhibit efflux ABC transporters or enzymes important for DOX disposition.[30] It might be that LIPDOX is distributed into liver regions and/or other tissues with higher metabolic capacity by AKRs and CBRs (i.e., different cell types, liver tumor tissue, cirrhotic liver tissue, normal liver tissue). Liver and kidney cells normally have the highest capacity (highest intrinsic clearance) for metabolizing DOX, while other organs have a lower capacity.[38,45] To our knowledge, there are no data yet available on the metabolic capacity of DOX in cirrhotic liver tissue or tumor tissue in HCC patients. CYP enzymes that are important for the metabolism of many other drugs are generally down-regulated in the cirrhotic liver and HCC,[46] but there appears to be no specific data regarding the expression of CBR1 and AKRs in HCC patients.

The local bioavailability values for DOX obtained after 6 h suggest that 51% and 12% of the dose was transported out of the liver after LIPDOX and DEBDOX delivery, respectively. This result support that the microparticle-based DEBDOX has a more localized and slower drug release compared to the emulsion based LIPDOX formulation. In the liver, the RD fraction of DOX may still reside in the intrahepatic DDS and/or be distributed into different compartments of the liver (i.e., extra- or intracellular space in tumor or nontumor liver tissue and bile). DOX that has entered the various liver and tumor cells could exert its cytotoxic effect, bind unspecifically to the cell interior, undergo metabolism, and/or be transported across the canalicular membrane. As LIPDOX is an emulsion that can readily separate on contact with the bloodstream, it is unlikely that DOX will still reside in LIPDOX after 6 h.[16] Instead DOX may have been distributed to tumor and nontumor liver tissue. A slow, prolonged release of DOX has been shown for DEBDOX both *in vitro* and *in vivo*, which implies a strong interaction between DOX and the DDS.[24,37,47−51] It is therefore likely that most of the dose will still reside within DEBDOX after 6 h. A foreign body response to the beads themselves has been reported to leave fibrous capsules around the beads after one month.[50] Therefore, it is questionable whether the total dose loaded will be completely released.[9,15] However, in healthy pigs, DEBDOX released 48% of the dose over 28 days and 89% over 90 days.[51] The fixed position of DEBDOX during the release and the static environment caused by embolization are likely to restrict released DOX from spreading to the circulation. This may be an important explanation for the low incidence of systemic adverse events. The bioavailability data clearly suggest differences in the release of DOX and its subsequent local distribution from these two DDSs. The clinical consequences of the slower onset of chemotherapeutic effect remain to be investigated and understood.

Of the patients included in this study, LIPDOX treatment resulted in a higher frequency ($n = 2$) of severe adverse events, i.e., liver failure. The subsequent death of one patient was probably related to alcohol intoxication by the patient shortly after their release from the hospital. There was no sign of liver failure in the DEBDOX group. There are not enough data to support whether the liver failure is a consequence of ischemia induced by the treatment or by the drug delivery from LIPDOX. The frequency of postembolization syndrome (nausea, fever, and abdominal pain) was similar between the groups. One month after the treatment, the overall response from the treatment was somewhat higher not significant) for DEBDOX than for LIPDOX; however, the two treatments had similar disease control.

There are limitations with this study to evaluate and compare the efficacy and safety between these DDSs. However, these limitations are not only valid for this study, but also for any assessment of clinical outcome for this treatment in other studies or in general. These limitations include differences in the degree of embolization achieved, the sizes of DEBDOX applied, the selectivity of the placement of catheter for administration, the intrahepatic distribution of the DDS, the local drug concentrations (not normalized), and the release rate of DOX from the DDSs and the size of the target lesion(s). In particular, there is a challenge involved in interpreting the safety and effect data because of the different degree of embolization achieved and from the different doses used in this clinical study (LIPDOX, all 50 mg of DOX; DEBDOX range 22.5−150 mg). Although the dose was normalized for the PK analysis, it is not possible to correct the safety and effect outcomes mathematically. Depending on how much of the effect that depends on the ischemia and/or on the drug (DOX) itself, both safety and effect will be dependent on the dose since DOX has a broad toxicity affecting all dividing cells. Since DEBDOX causes complete embolization, it has a necrotic effect in itself. Unloaded beads have been claimed to provide the same necrotic effect as DEBDOX, but DEBDOX contributes to a longer time to progression.[52] Therefore, the short-term necrotic effect on the main tumor after a single treatment is expected to be better for DEBDOX than for LIPDOX as a consequence of the superselective delivery and the complete embolization. However, LIPDOX and DEBDOX have different treatment strategies. With LIPDOX, the aim is to treat the same tumor and surrounding satellites during several repeated treatment sessions.[29] With DEBDOX, there is an opportunity to treat a single tumor in one course of treatment and, if the tumor has a partial response, the treatment can then be repeated from another vessel.[20,27] Future studies aiming to establish safety−benefit differences between these two DDSs need to follow the patients for a longer period and consider differences in dosage regimens.

In conclusion, LIPDOX releases DOX faster than DEBDOX in HCC patients and provides more extensive local and systemic exposure to both DOX and DOXol over 7 days. The poor stability of LIPDOX and the high systemic exposure (AUC and C_{max}) to DOX released from this DDS compared to DEBDOX are pharmaceutical drawbacks. Tumor and liver exposure to DOX is higher initially with LIPDOX, with the potential accumulation of lipiodol (and possibly DOX) within the tumor. The release of DOX from DEBDOX, however, is more sustained. DEBDOX has a release and distribution of DOX that is more controlled than LIPDOX, but more studies of the long-term release of DOX from DEBDOX in the diseased liver microenvironment are still required. This oncological indication remains in need of better treatment options for the delivery of drug(s) that may extend survival times for patients, and the challenge to develop more efficient DDSs to this end remains.

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AUTHOR INFORMATION

Corresponding Author

*Tel: +46-18 471 4317. Fax: +46-18 471 4223. E-mail: hans.lennernas@farmaci.uu.se.

ORCID

Elsa Lilienberg: 0000-0003-0271-4536

Erik Sjögren: 0000-0003-4318-6039

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AUC, area under the concentration−time curve; C_{max}, maximum concentration; DEBDOX, drug-eluting beads loaded with doxorubicin; DDS, drug-delivery system; DOX, doxorubicin; DOXol, doxorubicinol; *F*, bioavailability; f_e, fraction excreted to urine; HCC, hepatocellular carcinoma; i.v., intravenous; LIPDOX, lipiodol-doxorubicin emulsion; LLOQ, lower limit of quantification; PK, pharmacokinetics; PVA, poly vinyl alcohol; RD, remaining dose; RSD, relative standard deviation; TACE, transarterial chemoembolization

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