Published in final edited form as:

J Vasc Interv Radiol. 2020 October ; 31(10): 1697–1705.e3. doi:10.1016/j.jvir.2020.01.026.

Sodium Cholate Bile Acid Stabilized Ferumoxytol-Doxorubicin-Lipiodol Emulsion for Transcatheter Arterial Chemoembolization of Hepatocellular Carcinoma

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Abstract

Purpose: To develop bile acid stabilized multimodal MRI and CT visible doxorubicin eluting Lipiodol emulsion for the transarterial chemoembolization of hepatocellular carcinoma (HCC).

Materials and Methods: Ferumoxytol, an FDA approved magnetic resonance imaging (MRI) visible iron oxide nanoparticle, was electrostatically complexed with doxorubicin (DOX). An amphiphilic bile acid, sodium cholate (SC), was used to form a stable dispersion of Ferumoxytol-DOX complex in Lipiodol emulsion. The properties of fabricated emulsion were characterized in various component ratios. DOX release kinetics were evaluated for the chemoembolization applications. Finally, *in vivo* multimodal MRI/CT imaging properties and potential therapeutic effects upon intra-arterial (IA) infusion bile acid stabilized Ferumoxytol-DOX-Lipiodol emulsion were evaluated in orthotopic McA-Rh7777 HCC rat models.

Results: DOX complexed with Ferumoxytol through electrostatic interaction. Amphiphilic sodium cholate bile acid at the interface between the aqueous Ferumoxytol-DOX complexes and Lipiodol enabled a sustained DOX release ($17.2 \pm 1.6\%$ at 24 hr) at an optimized component ratio. In McA Rh7777 rat HCC model, IA infused emulsion showed a significant contrast around tumor in both T2W MRI and CT images ($P = 0.044$). H&E and Prussian-blue staining confirmed the local deposition of IA infused SC bile acid stabilized emulsion in the tumor. The deposited emulsion induced significant increases in TUNEL positive cancer cell apoptosis compared to a group treated with the non-stabilized emulsion.

Conclusion: SC bile acid stabilized Ferumoxytol-DOX-Lipiodol emulsion demonstrated sustained drug release and multimodal MRI/CT imaging capabilities. The new Lipiodol based formulation may enhance the therapeutic efficacy of chemoembolization in HCC.

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The authors declare no competing financial interests

Keywords

transarterial chemoembolization; emulsion; Ferumoxytol; Lipiodol; hepatocellular carcinoma

INTRODUCTION

Since the early 1980s, Lipiodol-based transarterial chemoembolization with doxorubicin (DOX) has been used as the standard of care for comparative studies with other intra-arterial therapies (drug-eluting beads, radioembolization), or systemic chemotherapy (sorafenib) in intermediate or advanced HCC patients.[1] Due to its properties of radiopacity, plasticity, drug delivery and embolization, Lipiodol has been widely adapted.[2–4]

DOX and Lipiodol emulsion has been used for standard conventional transarterial chemoembolization and the mixture systematically results in a water-in-oil (W/O) emulsion. [5] However, the insolubility of DOX in Lipiodol emulsion is characterized by high polydispersity. Non-reproducible DOX droplets are separated very rapidly *in vivo* because of their poor stability. Lipiodol co-localization is used as a proxy to predict therapeutic response, as imaging of DOX distribution is not currently feasible clinically. However this assumption may be limited due to the instability of the emulsion.[6]

The purpose of this study was to develop an amphiphilic bile acid stabilized Ferumoxytol-DOX-Lipiodol emulsion for use in chemoembolization (Figure 1). DOX loading efficiency, release kinetics and multimodal MRI and CT imaging properties of the emulsion were investigated. Finally, *in vivo* multimodal imaging and potential therapeutic effects following IA infusion of the emulsion were demonstrated in orthotopic McA-Rh7777 HCC rats.

MATERIALS AND METHODS

Materials.

Feraheme® (Ferumoxytol; AMAG, Waltham, MA, USA) was purchased from AMAG. Lipiodol was purchased from Guerbet (France). Sodium cholate (SC) hydrate was purchased from Sigma-Aldrich (BioXtra, St. Louis, MO, USA). Doxorubicin (DOX) hydrochloride (LC Laboratories, Woburn, MA, USA) was purchased in salt form and dissolved in aqueous solution when needed.

Formation of Ferumoxytol-DOX complex.

Ferumoxytol and DOX were complexed by a mixing procedure as follows: aqueous Ferumoxytol solution and DOX solution were mixed via magnetic stirring at room temperature for 24 hours. The formed Ferumoxytol-DOX complexes were then separated by magnets and further purified by centrifugation at 20,000 rpm. The complexes were washed three times with deionized water and air-dried. To optimize component ratio, five different Ferumoxytol:DOX (w/w) ratios (1:0.6, 1:1, 1:5, 1:10, and 1:20) at a constant Ferumoxytol concentration, 50 µg/ml, were used to complex Ferumoxytol-DOX.

Formulation of SC stabilized Ferumoxytol-DOX complex-Lipiodol emulsion.

To formulate emulsion with Lipiodol and the Ferumoxytol-DOX complexes, the pre-formed Ferumoxytol-DOX complex aqueous solutions (1:1 Ferumoxytol:DOX, 50 µg/ml) were mixed with Lipiodol by 30 back-and-forth pump exchanges through a three-way stopcock (Figure E1A), a widely used method in clinical practices.[7]. All emulsions were prepared at a 1:5 aqueous to oil phase volume ratio. For testing the stabilizing effect of SC in the emulsion, various concentrations of SC (0–5 mg/ml) were added in the emulsion.

Characterization.

The morphologies of Ferumoxytol-DOX complexes were examined with FEI Tecnai Spirit G2 Transmission Electron Microscope (Thermo Fisher Scientific, Waltham, MA, USA). To examine the interaction between Ferumoxytol and DOX, zeta potentials of Ferumoxytol-DOX complexes were measured by Zetasizer Nano ZSP (Malvern Instruments, Malvern, UK). The complexed DOX amount and complex (loading) efficiency were evaluated with UV-vis spectroscopy with Microplate Reader HT synergy. The loading efficiency (%) was calculated as $100 \times (\text{Total DOX} - \text{Free DOX}) / \text{Total DOX}$. The complexed DOX amounts at a fixed Ferumoxytol amount were calculated as $(\text{Total DOX} - \text{Free DOX}) / \text{Total Ferumoxytol}$. For the emulsion, the obtained Ferumoxytol-DOX complex Lipiodol emulsions were imaged with an optical microscope (Olympus CKX41, Center Valley, PA, USA) at 3 and 15 minutes after initial mixing and analyzed for average particle size by ImageJ software. To detect time-dependent DOX dispersity in the emulsion, two emulsions (with 0.5 mg/ml SC and without SC) were scanned by Nikon A1R laser scanning confocal microscope (Nikon, Tokyo, Japan) 1 day and 7 days after formulation.

In vitro drug release.

In vitro DOX release behavior of conventional DOX-Lipiodol, Ferumoxytol-DOX-Lipiodol and SC stabilized Ferumoxytol-DOX-Lipiodol emulsions were evaluated. An aliquot of 0.6 mL of each emulsion (corresponding to 0.2 mg of DOX) was loaded into a dialysis bag (M.W. cut-off: 12–14 kDa; Thermo Fisher Scientific, Waltham, MA, USA). The tubes were immersed in 20mL of buffer solution (1x PBS; pH 7.4) and incubated in a shaker (37 °C) rotated at a speed of 150 rpm. At predetermined times (up to 30 days), 0.4 mL of buffer solution was collected and replaced with an equivalent volume of fresh buffer solution. The released amounts of DOX were analyzed using a microplate reader (BioTek synergy HT, Winooski, VT, USA) equipped with a fluorescence detector. The fluorescence of DOX was detected at wavelengths of 480 nm (excitation) and 560 nm (emission). The lower limit of quantification (LLOQ) of DOX was 0.10 µg/ml.

Cell viability assay.

10,000 McA-RH7777 cells per well were cultured on 96-well plates and allowed to grow for 24 hours. 12.5 µg of SC were added to the cells. At 24- and 72-hour time points after SC addition, CCK8 solution was added to each well. After 2-hour incubation, absorbance at OD₄₅₀ was measured to assess the cell viability. Measurements were normalized against controls consisting of cells grown in unaltered culture media. Studies were performed in triplicate.

***In vivo* transcatheter intra-arterial infusion and MRI/CT imaging in McA-Rh7777 rat HCC model.**

All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee. McA-Rh7777 rats (n=9) were generated by implanting McA-RH7777 hepatoma cells in the left lateral liver lobe during mini-laparotomy procedures in Sprague Dawley rats, as reported by others.[8] Tumors were allowed to grow for 7 days to a size of around ~5 mm in diameter. Tumor induction rate in the rats was 100%. After 7 days of tumor growth, PBS (n=3), SC stabilized Ferumoxyl-DOX-Lipiodol emulsion (50 µg/ml of Ferumoxyl and DOX, 0.5 mg/ml of SC in 150 µL of emulsion) (n=3) or non-stabilized emulsion (50 µg/ml of Ferumoxyl and DOX in 150 µL of emulsion) (n=3) was infused after catheterization of the left hepatic artery as following reports by others.[9, 10] Final dose of sodium cholate and DOX were 12.5 µg and 1.25 µg, respectively. MRI studies were performed using a Bruker 7.0T ClinScan high-field small animal MRI system with a commercial rat coil (Bruker Biospin). Body temperature was monitored continuously and controlled with a water-bed (SA Instruments, Stony Brook, NY). CT and T2-weighted MR images were collected pre- and 2 hr post-arterial infusion of the emulsions, and contrast-to-noise ratios (CNR) were measured as follows: CNR = difference in signal intensities in ROI and external region/standard deviation of signal in the background. MR scans were performed using a gradient-echo sequence with following parameters: TR/TE = 1,300/7.2 ms, 0.7 mm slice thickness, FOV 71 × 85 mm, 216 × 256 matrix, respiratory triggering with MRI-compatible small animal gating system (Model 1025, SA Instruments, Stony Brook, NY). X-ray CT studies were performed using a micro-CT imaging system (NanoPET/CT®, Mediso Ltd-Bioscan Inc., Hungary-US).

Histology and immunohistochemistry analysis.

Each rat was euthanized at 72 hr after intra-arterial infusion procedure. Tumor bearing liver tissues were harvested and fixed with 10% neutral formalin solution. Samples were submitted to the pathology core for H&E, TUNEL and Prussian blue staining with 5 µm slice thickness. Tumor-bearing tissues were characterized by epithelial cellular shape and trabecular growth pattern.[11] To quantify tumor necrosis rates, the ratio of the necrotic area to the tumor area was calculated using ImageJ software. All slides were analyzed using a TissueFAXS microscope (TissueGnostics GmbH, Vienna, Austria) and histological evaluations were assessed by pathology researchers with 10 years of training.

Data Analysis.

Data were presented as mean ± SD and analyzed in R Studio (statistical software). Mann-Whitney U Test was performed for two independent samples, and Kruskal-Wallis test was performed for three or more independent samples. $P < 0.05$ was considered statistically significant.

RESULTS

Preparation and characterization of Ferumoxytol-Doxorubicin complex

Each zeta potential of Ferumoxytol and DOX was -29.0 ± 1.7 mV and $+18.8 \pm 0.3$ mV, respectively. When the Ferumoxytol and DOX complexed together, an upward trend of zeta potential was observed in higher DOX to Ferumoxytol ratios, confirming the deposition of DOX onto Ferumoxytol through electrostatic interactions (Figure 2A and 2B). There was an optimal Ferumoxytol and DOX ratio for efficient DOX loading and complexation with Ferumoxytol. The Ferumoxytol-DOX complexes formed with 1:1 (Ferumoxytol:DOX w/w) showed the highest loading efficiency of DOX at $69.65 \pm 5.52\%$. When the DOX amounts increased higher than 1:1, the loading efficiency was decreased as shown in Table 1. The Ferumoxytol-DOX complexes with 1:1 weight ratio of Ferumoxytol:DOX was selected and used for a combination with Lipiodol. The typical formation of Ferumoxytol-DOX complexes at 1:1 of Ferumoxytol:DOX ratio was confirmed with TEM (Figure E2).

Preparation and characterization of sodium cholate bile acid stabilized Ferumoxytol-DOX complex-Lipiodol emulsion

After 30 times of back-and-forth pumping exchanges, an emulsion of the water-phase droplets containing Ferumoxytol-DOX complex in Lipiodol oil phase was initially generated regardless of SC addition. After 15 minutes post formulation, the emulsion without SC showed clear phase separation whereas the SC stabilized emulsion remained homogeneous (partial phase separation was noticeable after 3–4 weeks) (Figure E1B). With increasing concentrations of SC, decreasing droplet sizes were clearly visualized with optical microscope images (Figure 3A). The average aqueous droplet sizes of the emulsions measured at 3 and 15 minutes post mixing showed a decreasing trend with the addition of SC in accordance with the optical images (Figure 3B). The addition of SC (0.5 mg/ml) also decreased the size of Ferumoxytol-DOX droplets to 13.9 ± 3.7 μm . However, with the mechanical force of pumping exchanges (30 times), the droplet size generated with SC concentrations above 0.5 mg/ml (up-to 5.0 mg/ml) showed a saturated size range of about ~ 18 μm . Even within 15 min of post-mixing, the decreased droplet size was well-maintained in the emulsion formulated with a concentration range of 0.5 – 5.0 mg/ml of SC ($P = 0.5608$, $n = 32$) (Figure 3B). The small droplets were slightly merged within 4 weeks post-mixing but the majority of droplets were still observed (Figure E3).

Controlled drug release of DOX from sodium cholate bile acid stabilized Ferumoxytol-Doxorubicin complexes-Lipiodol emulsion

SC stabilized Ferumoxytol-DOX-Lipiodol emulsion kept its initial form and a strong red fluorescence signal from DOX was detected in small emulsion droplets (Figure 4A). In contrast, when SC was not added in the emulsion, the released DOX and the separation of Ferumoxytol-DOX aqueous and Lipiodol oil phase were observed with a weak fluorescence signal and stronger red signal outside the emulsion droplets within 1-day post formulation. To demonstrate sustained-DOX release from the SC stabilized emulsion, time dependent DOX release rates for conventional DOX-Lipiodol and SC stabilized Ferumoxytol-DOX-Lipiodol were also compared *in vitro* (Figure 4B). DOX released from SC stabilized Ferumoxytol-DOX-Lipiodol emulsion was sustained over significantly longer period than

observed for conventional DOX-Lipiodol emulsion. The conventional DOX-Lipiodol emulsion released 93 % of DOX at 24 hr but only 17.2 % of DOX was released from SC stabilized Ferumoxytol-DOX-Lipiodol emulsion at 24 hr ($P < 0.0001$). The amount of drug released at 24 hr and 240 hr were also lower in the SC stabilized group than the non-stabilized Ferumoxytol-DOX group (respectively $P = 0.029$, $P = 0.008$).

In vivo Multimodal MRI and CT Image-guided Transarterial Chemoembolization procedure

In vitro MRI T2 images of SC stabilized Ferumoxytol-DOX-Lipiodol emulsions agreed with the stable dispersion of Ferumoxytol-DOX aqueous droplets in optical microscope observation: MRI T2 slice images at top and bottom of samples confirmed the Ferumoxytol-DOX complexes were stably dispersed in a whole area of sample (Figure E4). Equivalent IA delivery of the emulsions for non-stabilized and SC-stabilized groups was confirmed by CT contrast enhancement of Lipiodol (16.6 ± 1.9 vs 18.1 ± 3.0 , $P = 0.667$). (Figure 5A). Quantitative measurements of CT tumor contrast-to-noise ratio (CNR) of SC stabilized and non-stabilized emulsion post-infusion showed a statistically significant difference in contrast ($P = 0.029$) (Figure 5C). The tumor-localized DOX complexed with Ferumoxytol reduced T2 signal in MRI T2-weighted images (Figure 5B). The CNR change rate of SC stabilized Ferumoxytol-DOX-Lipiodol emulsion was significantly higher than the non-stabilized Ferumoxytol-DOX-Lipiodol emulsion ($P = 0.044$) (Figure 5D).

Histology

The tumor necrosis rate was $64.4 \pm 5.8\%$ in the group treated with SC stabilized Ferumoxytol-DOX-Lipiodol and $36.6 \pm 6.0\%$ in the group treated with non-stabilized emulsion ($P < 0.01$). The histological evaluation of iron presence (Ferumoxytol) using the H&E and Prussian-blue staining confirmed stronger blue signal around the tumor region in the group treated with IA infusion of SC stabilized Ferumoxytol-DOX-Lipiodol emulsion compared to the non-stabilized emulsion (Figure 6A and 6B), indicating a higher retention of Ferumoxytol-DOX complex. H&E and TUNEL stained tissue analysis (Figure 6A and 6C) displayed larger apoptosis area in the tissue infused with SC stabilized Ferumoxytol-DOX-Lipiodol emulsion than in the non-stabilized emulsion group, indicating that the higher DOX retention can induce increased cancer cell apoptosis. TUNEL-staining revealed significantly higher number of apoptotic cells in the group with SC stabilized emulsion compared with the non-stabilized emulsion ($P < 0.001$) (Figure 6D).

DISCUSSION

Conventional DOX-Lipiodol agent for transarterial chemoembolization was successfully reformulated with two biocompatible agents of Ferumoxytol and SC. An FDA approved agent Ferumoxytol is structured with iron oxide nanoparticle core and carboxymethyl-dextran surface. The carboxymethyl-dextran matrix can be utilized to carry one or multiple drugs within Ferumoxytol.[12] The electrostatic interaction between the carboxyl group on the surface of Ferumoxytol and the amino group of DOX allowed convenient complexation of the two components.

To combine Ferumoxytol-DOX complex into Lipiodol, biocompatible amphiphilic sodium cholate (SC) bile acid molecules were used. Bile acid-derived SC is amphiphilic, its hydrophilic face given by three hydroxyl groups and its hydrophobic face given by methyl groups on the steroid rings.[13] SC decreases interfacial tension between the polar and nonpolar phases, and allows formation of smaller droplets of the dispersed phase, as known in Young-Laplace equation ($\Delta p = 2\gamma/R$, Δp : pressure difference between the interface, γ : surface tension, R : radius of the curvature). When the Ferumoxytol-DOX complex was mixed with Lipiodol using a method practiced in clinic for formulating conventional DOX-Lipiodol emulsions, the addition of SC bile acid readily stabilized the dispersion of Ferumoxytol-DOX complex aqueous droplets in Lipiodol and the resulting emulsion was significantly more stable than the emulsions formulated without the addition of the bile acid. The conventional DOX-Lipiodol emulsion (Figure E5) and the Ferumoxytol-DOX-Lipiodol emulsion were thermodynamically unstable, and were characterized by wide size distribution of the dispersed aqueous droplets, which quickly aggregated and merged into two separate phases.

Subsequently, Ferumoxytol-DOX complex droplets with SC bile acid in Lipiodol resulted in enhanced DOX loading efficiency, sustained DOX release and MRI visibility of DOX. Sustained release of chemotherapeutic drugs from the embolic matrix can increase the concentration of the drug around the tumor for a longer period while lowering the systematic concentration, inducing ischemia and greater necrosis of the cancer cells.[14] Therefore, it is associated with lessening the typical side effects such as marrow suppression and cardiomyopathy in patients who received conventional chemoembolization.[15] Likewise in the study, the enhanced loading efficiency, sustained release and stability of DOX in bile acid stabilized Lipiodol could have attributed to better *in vivo* cancer cell killing effects by retaining DOX with Lipiodol longer within tumor region after IA infusion.

The SC bile acid stabilized Ferumoxytol-DOX-Lipiodol emulsion allowed us to monitor both Ferumoxytol-DOX and Lipiodol by MRI and CT, respectively. The bimodal imaging was important to track both DOX and Lipiodol, as their physical properties such as viscosity are different; the distribution of the drug and the embolic agent in the tissue may differ during the phase separation and it becomes difficult to predict the spread of drug with only CT tracking of Lipiodol, or vice versa. MRI-visibility of drug distribution co-localized with Lipiodol may provide more direct basis of assessing residual tumor (histological evaluations confirmed higher deposition of DOX with higher necrotic tumor tissues).

This work demonstrated MRI/CT visibility and short-term therapeutic effects from the novel chemoembolic agent, but additional *in vivo* studies are warranted to confirm dose-dependent long-term therapeutic effects and the survivability of the treated animal population. These studies would aid in delineating the clinical effect of DOX in hepatic embolization when the drug is retained for a longer time around the tumor than the conventional treatment. The multimodal CT and MR imaging properties of SC stabilized Ferumoxytol-DOX-Lipiodol emulsion will play an important role in evaluating whether the IA infused agents are well targeted on the tumor region and in predicting the therapeutic outcome of transarterial chemoembolization.

This study was a preliminary investigation into a proof of concept and thus had several limitations. The *in vivo* experiment used a reduced number of rats in order to confirm the bimodal imaging of the chemoembolic agent, localization of DOX, and the therapeutic effect of the agent. To more accurately assess the therapeutic benefits, higher number of animals should be used.

The viability of SC was tested to be non-toxic for a short-term period (Figure E6), but should be analyzed for a longer time to evaluate the long-term safety. Further, the therapeutic efficacy of the bile acid stabilized emulsion was evaluated by a qualitative confirmation of higher deposition of DOX and greater apoptosis around the tumor through histological examinations, but can be improved with quantification of intra-tumoral DOX concentration using mass spectroscopy.

In conclusion, SC stabilized Ferumoxytol-DOX-Lipiodol emulsion can offer a promising new chemoembolization agent for the treatment of HCC: the improved drug release profile of SC stabilized Ferumoxytol-DOX-Lipiodol emulsion allows a more sustained exposure of the treated tumor tissues which may contribute to greater treatment efficacy. The emulsion would be also ideal for selective IA administration to liver tumors while permitting MRI and CT visualization for patient-specific confirmation of tumor-targeted delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by grants R01CA218659 and R01EB026207 from the National Cancer Institute and National Institute of Biomedical Imaging and Bioengineering. This work was also supported by the Center for Translational Imaging and Mouse Histology and Phenotyping Laboratory at Northwestern University.

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Figure 1.

Schematic illustration of developing sodium cholate (SC) bile acid stabilized Ferumoxytol-Doxorubicin (Ferumoxytol-DOX) complexes-Lipiodol emulsion. Ferumoxytol was used as an MRI contrast agent and complexed with DOX by the electrostatic interaction between the negative surface of Feru and positively charged DOX. Amphiphilic SC molecules were used to generate a stable dispersion of Feru-DOX complex in Lipiodol for a chemoembolization agent.

Figure 2.

Ferumoxytol and doxorubicin (DOX) formed a complex in an aqueous solution (pH 7.4).

(A) A digital image of Ferumoxytol-DOX complex solution in different Ferumoxytol:DOX weight ratios. (B) Zeta potential of Ferumoxytol, DOX, and complexes at different ratios of Ferumoxytol:DOX. Data are presented as mean $\pm$ S.D.

Figure 3. Sodium cholate (SC) stabilizes Ferumoxytol-Doxorubicin (DOX)-Lipiodol emulsion between Lipiodol oil phase and Ferumoxytol-DOX complex aqueous phase. (A) Optical microscope images of Ferumoxytol-DOX-Lipiodol emulsion and SC stabilized Ferumoxytol-DOX-Lipiodol emulsion at different SC concentrations. Scale bar is 50 μm . (B) Average droplet size of Ferumoxytol-DOX in Lipiodol with different SC amounts. Data are presented as mean $\pm$ S.D.

Figure 4.

(A) Confocal images of emulsions taken at 1 hour, 1-day, and 7-day time points with and without sodium cholate. Sodium cholate concentration is 0.5 mg/ml. Scale bar is 50 μ m. Quick release of DOX in non-stabilized emulsion was marked by presence of red fluorescent signal outside the emulsion droplets, whereas SC stabilized emulsion droplets retained their signal. (B) *In vitro* release of DOX from the samples (DOX-Lipiodol, non-stabilized Ferumoxitol-DOX-Lipiodol and SC stabilized Ferumoxitol-DOX-Lipiodol. The SC stabilized group had more sustained release than the non-stabilized and DOX-Lipiodol group. The conventional DOX-Lipiodol group was characterized by initial burst release. Data are presented as mean $\pm$ S.D.

Figure 5.

(A) CT and (B) MRI T2-weighted images at pre- and 2 hr-post-intra-arterial infusion of sodium cholate stabilized Ferumoxetyl-DOX-Lipiodol emulsion and non-stabilized Ferumoxetyl-DOX-Lipiodol emulsion. Orange colored-dash-circles indicate the location of tumor. (C) CT contrast-to-noise ratio (CNR) of SC stabilized Ferumoxetyl-DOX-Lipiodol emulsion and the non-stabilized emulsion post-IA infusion ($P = 0.029$). (D) MRI contrast-to-noise ratio (CNR) change rates of SC stabilized Ferumoxetyl-DOX-Lipiodol emulsion and the non-stabilized Ferumoxetyl-DOX-Lipiodol emulsion both pre- and post-infusion ($P = 0.044$).

Figure 6.

Histological examination of tumor-bearing liver tissues treated with IA infusion of SC stabilized Ferumoxytol-DOX-Lipiodol emulsion or non-stabilized Ferumoxytol-DOX-Lipiodol emulsion. Representative images of (A) H&E, (B) Prussian blue, and (C) TUNEL stained tumor bearing liver tissues treated with IA infusion of SC stabilized Ferumoxytol-DOX-Lipiodol emulsion (top) or non-stabilized Ferumoxytol-DOX-Lipiodol emulsion (bottom) at 3 days post-IA infusion. (D) Quantification of TUNEL-positive cells for SC-stabilized Ferumoxytol-DOX-Lipiodol emulsion (SC+) and non-stabilized emulsion (SC-). TUNEL-positive cells were calculated within a $9.750 \times 10^4 \mu\text{m}^2$ field. Data are presented as mean $\pm$ S.D.

Table 1.

Loading Characteristics of Doxorubicin onto Ferumoxytol

Feru : DOX (w/w)	Complexed DOX Amount (DOX/Feru)	DOX Loading Efficiency (%)
1:0.6	0.42 ± 0.00	69.20 ± 0.52
1:1	0.70 ± 0.06	69.65 ± 5.52
1:5	1.26 ± 0.14	25.27 ± 2.79
1:10	1.24 ± 0.17	12.40 ± 1.74
1:20	1.26 ± 0.01	6.29 ± 0.06

Note – Data are presented as mean ± S.D.

Feru = ferumoxytol; DOX = doxorubicin hydrochloride.