Transarterial Chemoembolization with Doxorubicin; Drug Delivery, Pharmacokinetics and Tumor Response

BY

JOSEPH DOYLE MORRISON
B.S., University of Illinois, 2011
M.A., Loyola University Chicago, 2012
M.D., University of Illinois at Chicago College of Medicine, 2018

THESIS

Submitted as partial fulfillment of the requirements
for the degree of Master of Science in Clinical and Translational Science
in the Graduate College of the
University of Illinois at Chicago, 2018

Chicago, Illinois

Defense Committee:

Jack Zwanziger, Chair and Advisor
Ron C. Gaba, Interventional Radiology
Lawrence Schook, Animal Sciences, UIUC

ACKNOWLEDGEMENTS

The success of this project would not have been possible without Dr. Gaba and his dedicated involvement, guidance and patience throughout the process. This has truly been a learning experience to the fullest. My committee members- Dr. Jack Zwanziger and Dr. Lawrence Schook- have provided directed advice and opened my mind to a new way of thinking. To my research partner, Ramzy Khabbaz, without his help in this process it may have proved almost intolerable and overwhelming. To Dr. Lokken, who's help was critical to the proper execution of this study. The ultimate success of this project depended on these mentors and friends.

The Biologic Resources Laboratory (BRL) was critical for the daily operations of this project. Their patience and good humor allowed this to happen. The efforts of Ruth Muchiri and her team at Oregon State carried this across the finish line in the 11th hour. I would also like to thank the members of the department of Interventional Radiology for their support and assistance in this project.

JDM

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

LIST OF ABBREVIATIONS

AUC - Area under the concentration versus time curve

c-TACE - Conventional transarterial chemoembolization

DEE-TACE - Drug eluting embolic transarterial chemoembolization.

DOX - Doxorubicin

HCC - Hepatocellular carcinoma

HLBP – Hydrophilic lipophilic balance

HPLC-MS/MS or LC-MS/MS – High performance liquid chromatography tandem mass Spectrometry

IR - Interventional radiology

LRT - Locoregional therapies

mM – millimolar

mg – milligram

mL – milliliter

min – minute

ng – nanogram

μL – microliter

μg – microgram

RPMI – Roswell Park Memorial Institute Medium

TACE - transarterial chemoembolization

SUMMARY

Transarterial chemoembolization (TACE) selectively delivers chemotherapeutics to tumor arterial supply by way of a catheter introduced to the femoral artery and directed to hepatic arterial circulation (specifically in the case of hepatocellular carcinoma (HCC)). Chemotherapy is carried to end arterial feeders and immediate downstream portal sinusoids by various embolic materials. The combination allows for ischemic and toxic insults to promote tumor necrosis while relatively sparing the surrounding parenchyma. The gold standard drug delivery modality for TACE is ethiodized oil (Lipiodol; Gubert, Villepente France) and has been in use since TACE development in the early 1980's. This is known as conventional TACE (c-TACE) and enjoys a long history of good clinical efficacy. As novel drug delivery modalities were developed Lipiodol has served as the benchmark by which clinical efficacy is measured, but the tumoral uptake, concentration distribution and systemic pharmacokinetics of drug after c-TACE has not endured the same level of scrutiny as novel drug vectors to which it is being held in comparison. This study leveraged available modern chemical analytic techniques to evaluate tumoral drug temporal retention as well as systemic chemotherapeutic pharmacokinetics in an HCC animal model. Results bring c-TACE analysis abreast of modern techniques, contributing valuable support to current literature allowing for deeper understanding and continued progress.

In this Animal Care and Use approved study, 10 New Zealand white rabbit VX2 liver tumors were analyzed after receiving doxorubicin (DOX) c-TACE. Blood draws for systemic pharmacokinetic analysis were done pretreatment and at 2, 5, 10, 15, 20, 30, 45, 60, 120 and 180 minutes post treatment as well as at 1, 2, 4 and 7 days or until time of sacrifice. Animal sacrifice

was done at 180 minutes and then at 1, 2, 4 and 7 days (n=2/time point). Plasma and tumor samples were analyzed with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS or LC-MS/MS). Standard pharmacokinetic measures, including peak concentration (Cmax), half-life, and area under the concentration versus time curve (AUC), were performed to define intra-tumoral DOX retention dynamics and systemic pharmacokinetic profiles. These results were compared between VX2 tumor tissue and normal liver parenchyma and across time points using the paired samples T-test and one way analysis of variance (ANOVA).

Results of the current study show doxorubicin is retained in tumor tissue at 7 days after c-TACE at therapeutic levels while being undetectable in systemic plasma samples. These results bolster c-TACE as an effective embolic drug delivery medium while identifying areas of necessary progress.

I. INTRODUCTION

A. Background

Hepatocellular carcinoma (HCC) is the fifth most common cancer globally and the third most common cause of cancer-related death worldwide, accounting for more than 600,000 deaths each year. The incidence of HCC in the United States (US) is projected to increase over the next two decades, with more than 42,000 new diagnoses and 30,000 deaths expected in 2018[1]. Hepatocellular carcinoma is an insidious malignancy, often discovered after the opportunity for curative surgical resection has passed. Only 15% of liver cancers are considered curable via partial hepatectomy or liver transplantation; in most cases, underlying liver disease, size and/or number of tumors, tumor location, patient performance status, or transplant organ availability prevent surgery from being a viable therapeutic option. Thus, 85% of HCC patients must look to other therapeutic options for treatment, namely Interventional Radiology (IR) locoregional therapies (LRTs), defined as minimally invasive, image-guided ablative or arterially directed therapies[2].

Transarterial chemoembolization (TACE) is the prototypical LRT, and represents a widely recognized and globally employed treatment for HCC with proven survival benefit[3-6]. To this end, National Comprehensive Cancer Network (NCCN) guidelines recommend TACE as best management of confirmed HCC when it has been deemed unresectable, potentially resectable, transplantable, or inoperable due to performance status or comorbidity[7]. During the minimally invasive TACE procedure, IR physicians place a catheter into a branch of the hepatic artery (which is the predominant blood supply to HCC), and then deliver an embolic chemotherapeutic agent emulsion to vessels feeding tumor, spurring cancer necrosis through a combination of cytotoxicity from the chemotherapy, as well as ischemia from the mixture occluding end arterial branches,

thereby interrupting tumor blood supply. In conventional c-TACE—which is considered the standard form of therapy—the cytotoxic chemotherapeutic drugs are delivered directly to tumors as an emulsion with Lipiodol (Guerbet, Villepinte France), a radiopaque agent composed of di-iodinated ethyl esters of fatty acids from poppy seed oil, often followed by bland embolization inducing stasis in target vessels and preventing washout of the material[8 9]. This oil serves as both a carrier for chemotherapy drugs as well as an embolic agent that occludes tumor blood vessels and reduces tumor perfusion, increasing contact time between tumor cells and cytotoxic drugs.

B. <u>Study Rational</u>

Despite a long history of excellent clinical efficacy, preparation of a Lipiodol-DOX emulsion inherently lacks standardization of chemotherapeutic payload and lipid droplet size [3 8 9]. These differences lead to inconsistent results between investigators and patients[8]. To overcome dosage and droplet size discrepancies drug eluting embolics (DEEs) were developed in the 2000s allowing for a standardized and reproducible amount of drug to be loaded and delivered over an extend period[3]. Prolonged contact of tumor cells with cytotoxic levels of chemotherapy drugs is thought to enhance necrosis of TACE-treated tumors [8]. Due to their novelty, adoption of DEEs necessitated extensive preclinical/translational work on intra-tumoral drug distribution, penetration, and retention while c-TACE was not subjected to the same scrutiny, significantly limiting advancement of this procedure[9].

Pharmacokinetic studies of Lipiodol c-TACE are primarily limited to circulating drug levels[10] or indirect nuclear medicine based measurement techniques[11]. Very limited studies[12 13] have applied modern molecular imaging techniques, such as fluorescence imaging to identify the spatial distribution of chemotherapy drugs such as doxorubicin (DOX)- the most widely used

chemotherapy agent for TACE[14]- within tumor tissue and no contemporary studies have, to date, successfully identified the concentration distribution of compounds such as DOX in intact tissues after c-TACE. Delineation of systemic pharmacokinetics and concentration distributions of low molecular weight chemotherapeutic compounds in tumor tissue in a temporal manner after c-TACE is not only necessary to validate Lipiodol as a viable and effective drug delivery vehicle in the face of competing modern TACE techniques (i.e. DEE-TACE), but will also serve as a platform upon which to base future studies aimed at improving Lipiodol delivery of drugs and other therapeutic materials to liver tumors. Furthermore, an understanding of c-TACE drug delivery pharmacokinetics is imperative for elucidating the relative contributions of cytotoxic versus hypoxic cellular necrosis mechanisms in c-TACE (i.e. necrosis caused by chemotherapy versus necrosis caused by Lipiodol alone). Finally, clarifying intra-tumoral drug pharmacokinetics after c-TACE may provide a foundation for future comparison to other (e.g. nanoparticle) delivery methodologies.

C. Study Objective

The overarching, long-term intent of this research is to improve the prognosis of patients with surgically unresectable liver malignancies. The study here in accomplishes this end through delineating pharmacokinetic profiles of accepted locoregional drug delivery vehicles using chemical analytic techniques to lay foundations of understanding that may allow for progression of these essential therapies, ultimately resulting in improved over all survival and quality of life for the multitudes of affected individuals.

D. Significance and Innovation

The methodologies employed for this study represent significant and innovative approaches to facilitate therapeutic progression possibly affecting hundreds of thousands of patients with HCC per year. The significance of the proposed research lies in the characterization of intra-tumoral temporal retention of chemotherapeutic drug after c-TACE. Understanding gained contributes to improved HCC response to therapy and patient survival by providing vital pharmacokinetic information for comparing and optimizing TACE treatment protocols, allowing cytotoxic drug delivery and retention within tumors to be maximized. The innovation of the study lies in reconciling the early DOX tumoral and systemic pharmacokinetic underpinnings of c-TACE with modern analytic technologies. Findings allow comparison with competing interventions on a deeper level necessary for the fulminant maturation of this time proven approach. Further, establishing a foundation and scaffolding for comparisons in this manner only spurs on competition and fosters the adoption of the optimal treatment modality for the patient, which in this case is hundreds of thousands of people per year.

II. CONCEPTUAL FRAMEWORK AND RELATED LITERATURE

A. Conceptual Framework

This study is an analysis of c-TACE systemic pharmacokinetics and tumoral drug concentration and temporal retention using chemical analytic techniques to validate c-TACE as an efficacious chemotherapeutic delivery vehicle when compared to newer treatment modalities like DEE-TACE. The independent variable in the study is the intervention, c-TACE, and the dependent variables are the pharmacokinetic parameters derived from DOX plasma levels at various time points, as well as DOX's concentration within tumor tissue at similarly predetermined time points. Values of standard pharmacokinetic measures, including Cmax, half-life, and area under the concentration versus time curve (AUC) after c-TACE work to validate and deepen the understanding of c-TACE in the face of newer treatment options. Findings will be used to compare efficacy of c-TACE to novel techniques such as DEE-TACE to develop a framework for comparison of related treatment methodologies in the future.

B. Review of Related Literature

1. Transarterial Chemoembolization

Chemoembolization is the direct deposition of chemotherapeutics in the arterial bed supplying a tumor and subsequent embolization of the vessels, allowing prolonged cellular contact time with higher concentrations of the drug[2]. Localizing treatment to the arterial supply of tumors through peripheral access, such as femoral access, was started in the early 1980's[15 16] and was adopted due to observed tumoral response in the form of decreased size on computed tomography (CT), necrosis on histology, and decrease in the systemic tumor biomarker alpha-

fetoprotein[16]. Yet, superior efficacy to best supportive care was not observed until 2002[17] in a randomized control trial performed by Llovet et al[5] which was stopped early due to a statistically significant survival benefit seen in the TACE group. As such it has been embraced clinically for treatment of multinodular, noninvasive, asymptomatic HCC[18 19] and stands as the recommendation of the National Comprehensive Cancer Network for unresectable, potentially resectable or transplantable, or inoperable cancers due to performance status or comorbidity[7].

2. Lipiodol

a. Tumor Targeting

First developed in the 1980's, TACE has been dependent on Lipiodol for the vast majority of its clinical history and consequently Lipiodol serves as the gold standard for any comparative study with a novel TACE procedure[8]. Lipiodol provides a unique combination of radiopacity in conjunction with drug delivery and tumor seeking abilities[3 20 21]. The lipid medium favors larger vessel diameter when presented with divergent pathways and vessel hypertrophy, secondary to tumor hemodynamic demand, ensures Lipiodol preferentially follows tumor-feeding arteries[22]. In the tumor vasculature Lipiodol demonstrates what has been termed "plasticity" where by it adapts to fill terminal vessels of differing sizes, providing transient embolization of the feeding end arterioles as well as the portal sinusoids supplying the tumor[3 22 23]. This is clinically relevant because it helps prevent the development of collateral circulation[24] and it has been shown that extra capsular infiltrative tumor and surviving tissue on the perimeter are primarily supplied by portal sinusoids[25] where other drug delivery modalities do not display an embolic effect[26].

b. Tumor Uptake

Once Lipiodol has reached the tumor vasculature preferential absorption by human HCC cells has been seen[27]. This phenomenon was also reported in VX2 tumor, where a 1000-fold increase at 15 minuets and 100-fold increase at 3 days of Lipiodol concentration was seen for VX2 tissue when compared to other organs, supporting tropism for the VX2 tumor[28-31]. It has also been seen preferentially in the endothelium of tumor vessels[32]. Absorption in this manner may be an active process, but is primarily thought to be facilitated by pinocytosis[32] and cell-emulsion interface is increased by the relatively leaky vessels that supply HCC. When given alone the amount of Lipiodol retained in the necrotic areas of tumor on pathologic section has been seen to correlate with the amount of necrosis seen on CT[27 33 34] and survival time[35] suggesting an independent therapeutic effect[27]. Yet, other studies have disputed this correlation[36 37]. Ultimately uptake is influenced by a multitude of factors including the size of the tumor, the anatomy of the tumor microvasculature, the amount of tumor necrosis as well as blood pressure and permeability of the vasculature among other things[8].

c. Systemic Distribution

A 1984 study by Iwai et al.[31] mixed radio labeled C14 with Lipiodol and delivered it to the hepatic circulation of 63 rabbits harboring VX2 tumors in their livers. After delivery of the mixture through the hepatic artery, animals were sacrificed at 15 minutes, 3 days and 7 days. Sacrificed animals had various organs including, but not limited to the brain, lung, kidney, stomach, and intestine removed, minced and evaluated for radioactivity. Results showed the highest count for the tumor tissue with other organs and plasma showing 0.1% of the tumor tissue value at 15

minutes and 1% of tumor tissue value at day 3, suggesting good tropism for tumor tissue and brief if any retention systemically after administration. Systemic distribution is directly related to the size of the emulsion droplets delivered and the smaller the size the more likely they are to pass through the peribiliary plexus. In a rabbit VX2 model it was seen that the highest lung uptake was with pure lipiodol droplets of 10-40 μm[30]. While droplet sizes of 70-150 seem to display the most distal embolization while giving good drug release dynamics. In rats Lipiodol has been reported to be cleared from the portal vessels in 2-3 days and from the sinusoids in 7 days, at 15 days no oil was detected in the portal vein[38].

d. Tumor Retention

Extended contact with tumor is thought to enhance the necrotic effect of Lipiodol on HCC tumor tissue[8]. After c-TACE Lipiodol is actively and passively taken into tumor tissue[27 32] where it has been seen to be retained for 3 months post infusion[27] to as long as a year or more and the degree and duration of Lipiodol retention is directly correlated to the response of the tumor[24 39]. Retention is facilitated by the fact that there is no lymphatic drainage in HCC allowing increased tumor cell/Lipiodol emulsion interaction[40 41].

3. Chemotherapeutic

a. Emulsion

Most cytotoxic agents are hydrophilic, necessitating the creation of an emulsion to facilitate mixing with and delivery by Lipiodol. The ease of mixing is determined by the hydrophilic-lipophilic balance (HLB) of the molecule, representing the proportion of drug that is dissolved in the aqueous versus oily phase[8]. The preferential up take of Lipiodol by the tumor facilitates the

delivery of drug[42]. The highest carrying capacity is seen with water in oil emulsions meaning water is the discontinuous phase and oil is the continuous phase. This mixture prevents premature washout of the material while facilitating optimal drug delivery[42]. When Lipiodol is combined with a chemotherapeutic cytotoxic agent it is believed that there is a combined effect of ischemic and cytotoxic insult to the tumor[43]. Although the clinical value of this is contentious[5 14 44], including cytotoxic chemotherapeutics is recommended and considered standard of care[7]. Common drugs used in c-TACE are doxorubicin, epirubicin, mitomycin C, and cisplatin[14 45]. Chemotherapeutics can be given in combination or singly, and the most common drug in use is doxorubicin[14]. The exact dosage for chemotherapeutics are variable between providers and institutions though, with no standardization in place[8 14 21 45] and no evidence of superiority as it relates to overall survival has been seen for any one drug or combination[14].

b. Doxorubicin

Doxorubicin (DOX) is a nonselective class 1 anthracycline chemotherapeutic agent. This class of drugs inhibit enzymes of DNA replication allowing it to attack cells in any phase of the cell cycle, with a preference for mitotic cells[46 47]. Specifically DOX binds to DNA associated enzymes such as topoisomerase I and II while also intercalating with the base pairs of DNA[46] preventing cellular replication leading to apoptosis and cell death. Once delivered DOX has a distribution half-life of 3-5 minutes and a terminal half-life of 24-36 hours suggesting it is taken up by tissues very rapidly and takes far longer to be eliminated from those tissues[48]. DOX enters cells by passive diffusion to concentrations that exceed extracellular values by 10-500 fold and once in cells it concentrates in the nucleus at rates 50 fold higher than the cytoplasm[49]. Due to its broad

spectrum of anticancer activity and relatively clear spectral profile, DOX has been well suited for animal studies[12].

c. Pharmacokinetics

Lipiodol chemotherapeutic emulsions are generated to facilitate targeted delivery of the drug while limiting systemic side effects[8 11 13 50]. Once delivered 75% of Lipiodol has been seen to be taken up by the liver[51] and up to 40% of DOX is retained in Lipiodol at 3 hours[11]. When compared to strictly intra-arterial injection of DOX, a Lipiodol emulsion causes a significant decrease in the systemic levels of the drug detected at all time points and is significantly reduced further when followed by particle embolization[11 51]. Post c-TACE systemic levels of DOX have been seen to peak around 10-20 minutes[13 50] and reach levels of 180- 300 ng/ml as evaluated by LC-MS/MS[11-13].

d. Tumor Retention and Histologic Response

Lipiodol has been seen to increase the cytotoxicity of DOX [52] when combined with embolization of the feeding vessel leading to early extensive necrosis of the tumor reported at up to 90% at 3 days[50]. When given with Lipiodol intra-tumoral DOX has been shown to peak at ~ 60ng/mg[13] within tumor tissue and have a tumoral retention of ~ 3 days[11 13]. Yet, the uptake of DOX does not correlate with the uptake of Lipiodol[53] and no studies have demonstrated the concentration distribution of DOX in tumor tissue after c-TACE. Generally the relationship between the spatial-temporal distribution of chemotherapeutic and patient outcomes is tenuous and this information is critical to advancing c-TACE[9].

4. Liquid Chromatography-Tandem Mass Spectrometry

Mass spectrometry characterizes the steric arrangement of molecular components in a sample and has been the principle technique for identifying pharmaceutical compounds in a complex sample[54]. Liquid chromatography isolates compounds based on their electrostatic atmosphere. In complex samples such as tissue samples, obtaining appropriate resolution of target compounds through the noise is difficult for either technique individually. Coupling these techniques increases the signal to noise ratio while greatly reducing the processing time. For highly metabolized and active compounds such as DOX adding a tandem mass spectrometer allows dual analysis for metabolites of nearly identical structures further increasing the sensitivity and selectivity of processing[54]. Performing these analyses in conjunction is known as high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS or LC-MS/MS).

5. Animal Model

The VX2 tumor model has been extensively used since its development in the 1930's by Rous et al[55 56]. Since then this virus induced anaplastic squamous cell carcinoma modeled for a number of different tumor types including HCC[57]. Inherent characteristics such as easy propagation in skeletal muscle, rapid growth and hypervascularity make the tumor ideal for the clinical investigator on a short timeline, while the relatively large size of the rabbit vasculature and high growth rate make the system as a whole well adapted for interventional radiology procedures[57]. More recently, with the development of LRTs, this model has been employed in a number of studies looking at TACE procedures for HCC[58-60]. HCC is highly angiogenic[61] and microvessel density is associated with development and progression of the disease[62]. The high degree of angiogenesis in VX2 tumors give a good approximation for HCC in the regard. The

hypervascularity of the model, easy maintenance of New Zealand white rabbits and favorable hepatic anatomy make them a well-suited animal for this current investigation. Once the tumor develops in the hepatic lobe of implantation, hypertrophy of the primary hepatic artery feeder occurs. This allows good visualization of the tumor and reliable cannulation for direct chemoembolic delivery.

III. MATERIALS and METHODS

A. Design

This study was funded by a grant from Guerbet USA LLC. Animal Care and Use Committee approval was obtained for this prospective study (Protocol # 16-200). The protocol employs the rabbit VX2 tumor model, calling for use of disease free New Zealand white rabbits as experimental subjects. This model provides high tumor growth rate with adequately sized animal vasculature for catheterization and drug delivery[57]. The experimental protocol included: (a) injection of frozen or fresh anaplastic squamous cell VX2 tumor samples into the left hind limb of donor animals, (b) sacrifice and harvest of tumor from donor animals and surgical implantation into the left hepatic lobe of recipient rabbits, (c) in-vivo targeted intra-arterial delivery of DOX in a Lipiodol-water emulsion, (d) serum sampling after drug delivery, (e) animal sacrifice and harvest of tumor tissue, (f) chemical analysis of tumor tissue and systemic plasma samples to quantitatively compare local and systemic drug concentrations. The study included one treatment arm consisting of 10 rabbits undergoing c-TACE. Serum was acquired from each animal immediately prior to treatment and at 2, 5, 10, 15, 20, 30, 45, 60, 120 and 180 minutes as well as at 1, 2, 4 and 7days or until time of respective sacrifice. Animals were sacrificed at 180 minutes, 1 day, 2 days, 4 days and 7 days post intervention. The comparative temporal assessment of intra-tumoral DOX was designed to be a descriptive analysis utilizing 2 rabbits per time point across the 5 time points above. Use of these small sample sizes is consistent with pharmacokinetic studies in the literature[11-13 50].

B. VX2 Rabbit Tumor Model

1. Rabbit VX2 Tumor Cell Line Induction and Propagation

The VX2 cell line was originally obtained from frozen stock and propagated in a donor animal according to previously described methods[53 57]. Briefly, the VX2 tumor samples were defrosted for 4 minutes at 37 C after being stored in liquid nitrogen and then mixed with methylcellulose media in a 1:1 manner. The resulting mixture was then injected into the left quadriceps muscle group of a donor rabbit. After 2-3 weeks the donor animal was sacrificed, the tumor excised from the hind limb and transected. Several 1-2 mm pieces of tumor were selected for liver implantation. Viable tumor was then scraped from remaining specimen, collected, and strained in order to create a cell suspension using Roswell Park Memorial Institute (RPMI) medium (Sigma-Aldrich, St. Louis, MO USA) to wash the cells. Collected cells were spun in a centrifuge at 1,600 rotations per minute for 8 minutes, after which the supernatant was disposed of and the remaining cell pellet was resuspended in RPMI. Portion was mixed in methylcellulose media (Stemcell Technologies, British Columbia Canada) in a 1:1 ratio for immediate injection into the hind limb of another donor animal, maintaining the cycle. The remainder of the new sample was immediately stored in liquid nitrogen to replenish and maintain the cell stock.

2. Recipient Animal Liver Implantation

For liver tumor implantation surgeries, recipient rabbits were medicated with ketamine (30 mg/kg) and dexmeditomidine (0.1 mg/kg) for anesthetic induction, followed by intubation and maintenance with 1-3% isoflurane. One pre-procedure dose of enrofloxacin (5 mg/kg SQ) antibiotic prophylaxis was provided along with a 0.2 mg/kg loading dose of meloxicam. Bupivicane (<1mg/kg final total dose) was administered subcutaneously at the skin incision site.

Under aseptic conditions, a mini-laparotomy was performed in the subxiphoid area, exposing the liver. A scalpel was then used to make a stab wound approximately 5 mm deep in the liver parenchyma into which a 1-2 mm tumor fragment freshly harvested from donor rabbits was inserted. The wound was closed with a small (0.5 cm^2) piece of hemostatic surgical sponge (BloodSTOP iX; PRN Pharmacal, Pensacola FL). The abdomen was closed in two layers using 3-0 PDS suture (Ethicon, Somerville NJ) for fascial repair and 5-0 Vicryl suture (Ethicon) for cutaneous apposition. A 0.1 mg/kg meloxicam subcutaneous injection was given post procedure and for the subsequent three days. Post procedure, the animals were aroused and recovered, returned to cages, and monitored daily for wound healing and appetite until c-TACE. Liver tumors were incubated for 2-4 weeks prior to c-TACE based on previous experience of suitable 2-3 cm diameter tumor growth within 14-28 days[63].

C. Chemoembolic Preparation

1. DOX Lipiodol Emulsion

a. Dosimetry Rational

All c-TACE procedures were performed using an emulsion prepared with 0.2 mL DOX (McKesson, San Francisco CA) solution (0.2 mL of a 10 mg/mL DOX solution) and 0.4 mL Lipiodol mixed in a 1:2 volumetric ratio (0.6 mL total volume); a water in oil emulsion (1:2 ratio) has shown more optimal drug carriage capacity and release characteristics as compared to an oil in water emulsion (1:2 ratio)[42]. The use of DOX as the chemotherapeutic agent of interest is due to its widespread use as a monotherapy agent during TACE[64]. The 2 mg DOX dose proposed is empiric in nature, but doses in this range (1.25-3.75 mg) result in weight based doses ranging from 417-1,250 mcg/kg (assuming a typical rabbit weight approximating 3.0 kg), which—when

administered in a targeted fashion to liver only—have been associated with VX2 tumor necrosis in the published experience of the Principal Investigator [65] and lie below the systemic DOX intravenous lethal dose to 50% of animals (LD50) in rabbits (5,980 mcg/kg)[5]. Of note, no literature data on DOX cytotoxicity or lethality for VX2 cells were available to help devise the DOX dosing regimen, although DOX has shown antitumor effect in prior VX2 TACE studies[65-67].

b. Preparation

Lyophilized powder DOX was reconstituted in aqueous solution to create a 10 mg/mL solution. 0.2 mL of 10 mg/mL DOX aqueous solution was emulsified with 0.4 mL of Lipiodol alone, immediately prior to c-TACE administration using the Tessari-Tourbillon three-way stopcock technique[68]. Care was taken to ensure homogeneous emulsification of the chemotherapy and Lipiodol mixture at the time of administration by cycling the drug emulsion between two syringes connected by a three-way stopcock at least 20 times.

D. Conventional-Transarterial Chemoembolization Procedures

All pharmaceuticals and embolic devices used in c-TACE (eg. Lipiodol and DOX) were medical/pharmaceutical grade and prepared sterile by manufactures. For c-TACE procedures, rabbits were medicated for anesthetic induction with ketamine (30mg/kg) and dexmeditomidine (0.1 mg/kg), followed by intubation and maintenance with 1-3% isoflurane. In addition, they received a loading dose of 0.2 mg/kg meloxicam (Boehringer Ingelheim, Ingelheim Germany) subcutaneous injection and 5mg/kg enrofloxacin (Bayer, Laverkusen Germany) subcutaneous injection prior to the procedure. Bupivicaine (Abbott Laboratories, Abbott Park IL) <1mg/kg final total dose was administered subcutaneously at the skin incision site. Angiography was performed

with a C-arm unit (OEC Medical Systems series 9600; GE Healthcare, United Kingdom). The femoral artery was then accessed through a surgical cut-down and catheterized with a 3 French vascular sheath (Check-Flo Performer Introducer; Cook Medical, Bloomington IN), after which a 2.3 French microcatheter (Boston Scientific, Natick MA) was advanced over a guide wire and the celiac artery was selectively catheterized. Angiography of the common and proper hepatic arteries was then performed via injections of iohexol (Omnipaque-300; Amersham Health). After obtaining angiographic confirmation of microcatheter placement within the left hepatic artery, TACE was performed by injection of Lipiodol-DOX. Under fluoroscopic visualization, the Lipiodol emulsion was injected by hand. The anticipated endpoint of each TACE procedure was administration of the entire prescribed chemotherapy dose. After procedure completion, the catheter was removed, common femoral artery ligated using non-absorbable silk suture (Ethicon) to obtain hemostasis, and the groin incision was closed using Vicryl suture (Ethicon) in the subcuticular tissue and non-absorbable Ethilon nylon suture (Ethicon) in cutaneous tissue. After the procedure rabbits received 0.1 mg/kg meloxicam (Boehringer Ingelheim, Ingelheim Germany) subcutaneous injection for three days. Immediately post-procedure the animals were aroused, returned to cages, and monitored daily until time of sacrifice.

E. Sample Acquisition and Processing

1. Serum Sampling, Animal Necropsy and Tissue Harvest

For systemic DOX level measurement, a 2 mL sample of arterial blood was obtained from the femoral artery from each rabbit at 2, 5, 10, 15, 20, 30, 45, 60, 120, and 180-minutes post c-TACE, as well as from the central ear artery at 1, 2, 4, and 7-days post-procedure or until time of respective sacrifice. Rabbits were euthanized 180-minutes post-procedure and at 1, 2, 4, and 7-

days using a lethal dose of 150 mg/kg pentobarbital sodium solution (Schering-Plough, Kenilworth NJ). Two rabbits (with 1 tumor each) were sacrificed per time point (10 rabbits and 10 tumors total). Treated tumors were harvested and dissected from adjacent liver for processing. Sacrifice at these time points allowed for differential characterization of the intra-tumoral uptake of DOX after c-TACE. Findings covered an established detectable time period for systemic DOX levels post TACE[69].

2. Serum Sample Processing

Once collected blood samples were spun at 2,000 rotations per minute for 15 minutes. The resulting supernatant represented cell free serum of which .75ml was aspirated and deposited in a 1ml micronix tube(check) before being immediately placed in liquid nitrogen for storage at -80 °C prior to LC-MS/MS analysis. All LC-MS/MS analysis was done by a third party according to previously described techniques[54].

3. Histological Processing of Tissue Samples

Tumors were transected along the mid portion and half of the tumor was frozen in saline for LC-MS/MS analysis.

F. Analysis

1. Doxorubicin Quantification

a. Liquid Chromatography-Tandem Mass Spectrometry Quantification

Quantification of intra-tumoral and systemic DOX was performed according to previously described technique[53 70 71]. Processing was completed using a ScieX API 4000 mass spectrometry

machine (AB Sceix UK Limited, Cheshire UK) and transitions for the MRM method used in quantitation were: 544.0 > 397.0 (Doxorubicin), 548.1 > 401.1 (13 C-d 3 -Doxorubicin).

i. Tumor and Liver Tissues Preparation

Briefly, tissues were homogenized in phosphate buffer (0.05M, pH7.4) to produce a homogenate containing 0.2 grams of tissue per mL. Homogenized tissue was then extracted with four volumes of ice-cold acetonitrile before being centrifuged at 13,000g for 15 minutes. The supernatant of each was removed and dried in a speed vac before being reconstituted in 150µL of 50:50 acetonitrile and ultrapure water containing 5mM ammonium acetate attaining a pH of 3.5 and internal standard of 13 C-d 3 -Doxorubicin at 100 ng/ml. Then an aliquot of 5µL was injected into the mass spectrometer (ScieX API 4000) for analysis.

ii. Serum Sample Preparation

Serum samples were analyzed in duplicate. Briefly, 250µL of serum was added to 750µL of acetonitrile and vortex mixed for 4 minutes. The mixture was centrifuged at 13,000g for 15 minutes at 4 °C. The supernatant was then transferred to a clean micro-centrifuge tube, and dried down in the speed vac. Samples were reconstituted in 150µL of 50:50 acetonitrile and ultrapure water containing 5mM ammonium acetate, pH 3.5 and internal standard (13 C-d 3 -Doxorubicin at 100 ng/ml). Then an aliquot of 5µL was injected into the mass spectrometer (ScieX API 4000) for analysis.

2. <u>Drug Delivery Outcome Measures and Quantitative Analysis</u>

The primary drug delivery outcome measures of the current study were: (1) the durability of DOX delivery and systemic levels measured using standard pharmacokinetic measures, including C_{max}, half-life, and AUC, after c-TACE.

Intra-tumoral and systemic DOX levels quantified by HPLC-MS/MS were used for pharmacokinetic calculations. Half-life was calculated using the formula:

$$N_t = N_0 * (1/2)^{t/t1/2}$$

C_{max} was defined as the peak intra-tumoral DOX concentration. AUC was calculated using the linear trapezoidal method[72].

3. <u>Statistical Analysis</u>

Statistical analysis will be performed with SPSS version 22 when sufficient data has been collected. Pharmacokinetic measures will be compared between c-TACE treatment group time points as well as between tumor and normal liver parenchyma using paired samples t-test in conjunction with analysis of variance using the Bonferroni post hoc technique for comparison across time points. Results are expected to be of statistical significance, p-value of at least <0.05.

IV. RESULTS

Animal characteristics are summarized in Appendix A.

A. Procedure

A total of 12 rabbits underwent 12 hepatic implantations and 10 TACE procedures with ethiodized oil (n=12). For two animals (16.7%) no treatment was administered after no tumor was identified during the TACE procedure, which was subsequently confirmed on necropsy, despite direct hepatic implantation of VX2 tissue. These animals were excluded from the analysis. The remaining 10 animals underwent successful TACE procedures. Post procedure 2 (16.7%) animals had unilateral hind limb paralysis coinciding with femoral access site. Symptoms consisted of foot drop (n=1, 8.3%), and complete paralysis (n=1, 8.3%). These animals were sacrificed for the 4-day time point. Several rabbits (n=3, 25%) dislodged their abdominal sutures requiring reapproximation. Paralysis post procedure was thought to be due to prolonged vascular access (3 hours) with a 3-F sheath resulting in ischemic compromise of the catheterized extremity. Subsequently the decision was made to remove the vascular access after 1 hour and take remaining blood draws via a marginal artery of the ear.

B. Tissue Pharmacokinetics

Analysis of VX2 tissue post c-TACE was performed for tumor tissue and on the immediately adjacent normal liver parenchyma. For the animals sacrificed (n=2/time point) at 180-minutes, 1, 2, 4 and 7 days, DOX concentrations were seen in the tumor and liver tissue at 5,989.8 ± 1,063ng/mL and 173.5 ± 228ng/ml, 1,715.6 ± 1,951.4ng/ml and 194.2 ± 96.2ng/ml, 956.5 ± 657.6ng/ml and 45.7 ± 31.5ng/ml, 1,547 ± 620.8 ng/ml and 112.9 ± 86.2ng/ml, 216.9 ±80.6ng/ml and 7.5 ±1.3ng/ml respectively. The tumoral versus parenchymal DOX concentrations were

significantly different (P= 0.021) over all time points. The 180 minute time point had a significantly higher intra-tumoral concentration than the 1day (P=.011), 2 day (P=0.005) , 4 day (P=0.009) and 7 day (P=0.003) time points on post hoc analysis using the Bonferroni correction technique. Remaining samples showed a non-significant decline in DOX concentration, graphically demonstrated in Figure I, with a collective tumor to liver partition ratio of 19.56.

The Cmax of DOX in tumor tissue was seen at 6,741.5ng/mL for the 180-minute time point, compared to 334.7ng/mL in the adjacent normal parenchyma. DOX half-life and AUC for tumor tissue and live parenchyma were calculated at 2,106 minutes and 14,193μg•min/mL compared to 1,938 minutes and 893μg•min/mL respectively. All pharmacokinetic values can be seen in Table II.

C. <u>Plasma Pharmacokinetics</u>

The DOX plasma concentration was seen to peak 2 minutes after delivery at 581 ± 337ng/mL with a Cmax of 1,348.5ng/ml. Subsequently there was a steady decline over the 5, 10, 15, 20, 30, 45, 60, 120 and 180 minute time points showing 297.8 ± 142ng/ml, 110.4 ± 57.4ng/mL, 74.3 ± 52.7ng/mL, 37.3 ± 25.1ng/mL, 23.8 ± 10.2ng/mL, 16 ±10.3ng/mL, 13.1 ± 7.1ng/mL, 10.1 ± 6.9ng/mL and 8.2 ± 6.2ng/mL respectively. Plasma samples at 1 day, 2 days, 4 days and 7 days revealed DOX concentrations at 1 day (3.5 ± 4.3ng/mL) and 2 days (0.2 ± 0.5ng/mL), but fell to undetectable levels at the 4 and 7 day time points as seen in Figure 2. Half-life analysis of DOX revealed an average 149.5 ±124.8 minutes with an area under the concentration versus time curve of 15.67μg•min/mL and a tumor to plasma partition ratio of 20.69. Plasma pharmacokinetic parameters can be seen in Table II.

V. DISCUSSION

A. Discussion

Here we have shown that DOX is preferentially retained in HCC tissue at significant levels for up to 7 days post c-TACE. Systemic plasma levels peaked at 2 minutes after infusion and then fell significantly over the next 8 minutes before steadily decreasing to almost undetectable levels at 2 days and vanishing by 4 days post treatment, despite continued pharmacologically relevant concentrations persisting in tumoral tissue. Average tumor tissue DOX concentrations met or surpassed reported IC50 values[73] (levels sufficient to inhibit 50% of local cellular proliferation) at 6hrs (3,828ng/mL), 1 day (489.9ng/ml) and 3 days (223ng/mL) in all our samples and overall intra-tumoral concentrations of DOX were seen to be significantly higher (P=0.021) than the surrounding parenchyma. This was further demonstrated by a higher Cmax, area under the concentration versus time curve and longer half-life for DOX in tumor tissue compared to adjacent parenchyma and systemic plasma samples. Further, partition ratios for tumor to liver (19.56) and tumor to plasma (20.69) favored tumor tissue localization of DOX. In conjunction these results would seem to support preferential uptake of DOX to tumoral tissue over adjacent liver parenchyma, mirroring known Lipiodol behavior[27] despite concentrations not directly correlating[74].

Previously, early post c-TACE DOX systemic pharmacodynamics and tumoral retention profiles have not been evaluated to the extent reported here[11 12 26 50] and the findings have been contentious. In a 2017 study by Zhang et al.[12] no DOX was able to be detected in tumor tissue at 7 days, in line with a 2006 report by Hong et al.[50] and a 2011 report by Gupta et al.[13]. Hong did see intra-tumoral DOX at 3 days, yet levels were much lower than reported here despite an initial dose of 11.25mg DOX being delivered. Similarly, Gupta detected DOX concentrations in tumor tissue at

1 and 3 days after an initial dose of 4mg DOX, but levels were lower at 1 day (61±41ng/ml) than reported here at 4 days (956.5 ± 657.6ng/ml). These discrepancies may be accounted for by the fact that in both studies DOX was not emulsified in Lipiodol, but added directly, possibly allowing for early phase separation and washout. Alternatively, a 2014 report by Choi et al.[26] saw a much higher concentration at 7 days (6,750 ± 2473ng/ml) after an initial dose of 2.4mg DOX delivered as a water in oil emulsion. All studies did show similar systemic pharmacokinetic profiles though, with the peak concentration occurring at the earliest measured time point and levels falling to under 50ng/mL by 1hr.

The findings reported here, produced using clinically appropriate Lipiodol DOX emulsion ratios and concentrations, frame c-TACE DOX systemic and tumoral pharmacokinetics in a modern light. We saw that DOX is retained at higher levels and for longer than previously assumed after c-TACE, adding small but essential clarity to the behavior of chemotherapeutics in the in-vivo setting. From here the next step is to understand the actual concentration distribution of the drug in tumor and what relation it has to tumoral necrosis and then to compare these findings to DEE-TACE. The extended pharmacologic profile after DEE (mainly reduced systemic side effects) has been assumed relate to overall improved clinical efficacy, but this has not born out to the extent expected[75] and highlights the need for better understanding of the pharmacokinetic and tumoral drug retention profile after TACE procedures. In depth understanding of the systemic and tumoral pharmacokinetics for these interventions will allow for identification of areas of complement, further facilitating necrosis and increasing the efficacy of treatment overall. Possible future treatment profiles my garner a more extensive initial tumoral response by using Lipiodol DOX in conjunction with angiogenic inhibitors or followed by DEE-TACE to achieve stasis as opposed to bland tris-acryl microspheres. This study helps to build an understanding which may act as a

natural segue for more complete analysis of the basic elements which determine clinical efficacy, ideally leading to the ultimate optimization of all treatment modalities to provide improved outcomes for the patient.

B. Limitations

This study had several limitations. First, the sample size for the treatment groups was relatively small to generate appreciable power for our claims. Second, The VX2 tumor, although the preferred representation for HCC in this animal model, is an imperfect surrogate for human HCC which may result in differential tumor uptake patterns. Third, limitations of the animal model itself as it is an imperfect representation of true human metabolism and comorbidities.

C. Conclusion

In conclusion, results have shown DOX to be preferentially retained in HCC tumor tissue at pharmacologically relevant concentrations for at least seven days with peak systemic concentrations occurring 2 minutes after infusion and falling precipitously over the subsequent 3 hours post procedure, ultimately reaching negligible levels by 2 days. With these results further support is added to the foundations of TACE drug delivery understanding. Knowing differences in pharmacokinetic underpinnings of these treatment modalities allows for progression to the next step in understanding tumoral localization, temporal retention and percent necrosis attributable to TACE delivered drugs and their differences. Amalgamation of this information in conjunction with appropriate tailoring of our treatment modalities to optimize what is found will provide an improved result for the patient.

CITED LITERATURE

1. American Cancer Society. Cancer Facts and Figures 2018 [Available from: https://cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2018.html accessed January 10 2018.

2. Eastern Cooperative Oncology Group. ECOG E1208 Clinical Trial. accessed at http://ecog-acrin.org/clinical-trials/e1208-educational-materials.

3. Woo HY HJ. Transarterial chemoembolization using drug eluting beads for the treatment of hepatocellular carcinoma: Now and future. *Clinical and Molecular Hepatology* 2015;21(4):344.

4. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003;37(2):429-42. doi: 10.1053/jhep.2003.50047 [published Online First: 2003/01/24]

5. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002;359(9319):1734-9. doi: 10.1016/S0140-6736(02)08649-X [published Online First: 2002/06/07]

6. Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002;35(5):1164-71. doi: 10.1053/jhep.2002.33156 [published Online First: 2002/05/01]

7. Network NCC. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Hepatobiliary Cancers Version 1.2016, 2016.

8. Idée JM GB. Use of Lipiodol as a drug-delivery system for transcatheter arterial chemoembolization of hepatocellular carcinoma: a review. *Critical reviews in oncology/hematology* 2013;88(3):530-49.

9. Kilcup N, Tonkopi E, Abraham RJ, et al. Composition-property relationships for radiopaque composite materials: pre-loaded drug-eluting beads for transarterial chemoembolization. *J Biomater Appl* 2015;30(1):93-103. doi: 10.1177/0885328215572196 [published Online First: 2015/02/19]

10. Johnson PJ, Kalayci C, Dobbs N, et al. Pharmacokinetics and toxicity of intraarterial adriamycin for hepatocellular carcinoma: effect of coadministration of lipiodol. *J Hepatol* 1991;13(1):120-7. [published Online First: 1991/07/01]

11. Raoul JL, Heresbach D, Bretagne JF, et al. Chemoembolization of hepatocellular carcinomas. A study of the biodistribution and pharmacokinetics of doxorubicin. *Cancer* 1992;70(3):585-90. [published Online First: 1992/08/01]

12. Zhang S HC, Li Z, Yang Y, Bao T, Chen H, Zou Y, Song L. Comparison of pharmacokinetics and drug release in tissues after transarterial chemoembolization with doxorubicin using diverse lipiodol emulsions and CalliSpheres Beads in rabbit livers. *Drug Delivery* 2017;24(1):101-7.

13. Gupta S, Wright KC, Ensor J, et al. Hepatic arterial embolization with doxorubicin-loaded superabsorbent polymer microspheres in a rabbit liver tumor model. *Cardiovasc Intervent Radiol* 2011;34(5):1021-30. doi: 10.1007/s00270-011-0154-6 [published Online First: 2011/04/12]

14. Marelli L SR, Triantos C, Senzolo M, Cholongitas E, Davies N, Tibballs J, Meyer T, Patch DW, Burroughs AK. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. *Cardiovascular and interventional radiology* 2007;30(1)

15. Konno T, Maeda, H., Yokoyama, I., Iwai, K., Ogata, K., Tashiro, S., Uemura, K., Mochinaga, M., Watanabe, E., Nakakuma, K. and Morinaga, T. Use of a lipid lymphographic agent, lipiodol, as a carrier of high molecular weight antitumor agent, smancs, for hepatocellular carcinoma. *Cancer & chemotherapy* 1982;9(11):2005-15.

16. Konno T. Targeting cancer chemotherapeutic agents by use of Lipiodol contrast medium. *Cancer* 1990;66:1897-903.

17. Georgiades CS, Hong K, D'Angelo M, et al. Safety and efficacy of transarterial chemoembolization in patients with unresectable hepatocellular carcinoma and portal vein thrombosis. *J Vasc Interv Radiol* 2005;16(12):1653-9. doi: 10.1097/01.RVI.0000182185.47500.7A [published Online First: 2005/12/24]

18. European Association For The Study Of The L, European Organisation For R, Treatment Of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56(4):908-43. doi: 10.1016/j.jhep.2011.12.001 [published Online First: 2012/03/20]

19. Lencioni R, Crocetti L. Local-regional treatment of hepatocellular carcinoma. *Radiology* 2012;262(1):43-58. doi: 10.1148/radiol.11110144 [published Online First: 2011/12/23]

20. Liapi E GJ. Transcatheter arterial chemoembolization for liver cancer: is it time to distinguish conventional from drug-eluting chemoembolization? *Cardiovascular and interventional radiology* 2011;34(1):37-49.

21. SW S. The current practice of transarterial chemoembolization for the treatment of hepatocellular carcinoma. *Korean journal of radiology* 2009;10(5):425-34.

22. de Baere T, Dufaux J, Roche A, et al. Circulatory alterations induced by intra-arterial injection of iodized oil and emulsions of iodized oil and doxorubicin: experimental study. *Radiology* 1995;194(1):165-70. doi: 10.1148/radiology.194.1.7997545 [published Online First: 1995/01/01]

23. Kan Z. Dynamic study of iodized oil in the liver and blood supply to hepatic tumors. An experimental investigation in several animal species. *Acta Radiologica* 1996;37:7-24.

24. Imaeda T YY, Seki M, Goto H, Iinuma G, Kanematsu M, Mochizuki R, Doi H, Saji S, Shimokawa K. Lipiodol retention and massive necrosis after lipiodol-chemoembolization of hepatocellular carcinoma: correlation between computed tomography and histopathology. *Cardiovascular and interventional radiology* 1993;16(4):209-13.

25. Kan Z, Madoff DC. Liver anatomy: microcirculation of the liver. *Semin Intervent Radiol* 2008;25(2):77-85. doi: 10.1055/s-2008-1076685 [published Online First: 2008/06/01]

26. Choi JW CH, Park JH, Baek SY, Chung JW, Kim DD, Kim HC. Comparison of drug release and pharmacokinetics after transarterial chemoembolization using diverse lipiodol emulsions and drug-eluting beads. *PloS one* 2014;9(12):e115898.

27. Park C, Choi SI, Kim H, et al. Distribution of Lipiodol in hepatocellular carcinoma. *Liver* 1990;10(2):72-8. [published Online First: 1990/04/01]

28. Akashi Y, Koreeda C, Mizuno T, et al. Hepatic parenchymal changes after the intraarterial injection of lipiodol in tumor-bearing rabbits. *Invest Radiol* 1993;28(2):128-32. [published Online First: 1993/02/01]

29. Hamuro M, Nakamura K, Sakai Y, et al. New oily agents for targeting chemoembolization for hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 1999;22(2):130-4. [published Online First: 1999/03/30]

30. de Baere T, Zhang X, Aubert B, et al. Quantification of tumor uptake of iodized oils and emulsions of iodized oils: experimental study. *Radiology* 1996;201(3):731-5. doi: 10.1148/radiology.201.3.8939223 [published Online First: 1996/12/01]

31. K. Iwai HM, T. Konno. Use of oily contrast medium for selective drug targeting to tumor: enhanced therapeutic effect and X-ray image. *Cancer Research* 1984;44:2115-21.

32. Bhattacharya S, Dhillon AP, Winslet MC, et al. Human liver cancer cells and endothelial cells incorporate iodised oil. *Br J Cancer* 1996;73(7):877-81. [published Online First: 1996/04/01]

33. Kim SJ, Choi MS, Kang JY, et al. Prediction of complete necrosis of hepatocellular carcinoma treated with transarterial chemoembolization prior to liver transplantation. *Gut Liver* 2009;3(4):285-91. doi: 10.5009/gnl.2009.3.4.285 [published Online First: 2010/05/01]

34. Takayasu K, Arii S, Matsuo N, et al. Comparison of CT findings with resected specimens after chemoembolization with iodized oil for hepatocellular carcinoma. *AJR Am J Roentgenol* 2000;175(3):699-704. doi: 10.2214/ajr.175.3.1750699 [published Online First: 2000/08/23]

35. Monsky WL, Kim I, Loh S, et al. Semiautomated segmentation for volumetric analysis of intratumoral ethiodol uptake and subsequent tumor necrosis after chemoembolization. *AJR Am J Roentgenol* 2010;195(5):1220-30. doi: 10.2214/AJR.09.3964 [published Online First: 2010/10/23]

36. A. Ono IH, M. Ueno. Study on hepatic artery chemoembolization using temperature-sensitive liposome or Lipiodol emulsion. *Biological and Pharmaceutical Bulletin* 1995;18:279-83.

37. Pourgholami MH, Lu Y, Wang L, et al. Regression of Novikoff rat hepatocellular carcinoma following locoregional administration of a novel formulation of clofazimine in lipiodol. *Cancer Lett* 2004;207(1):37-47. doi: 10.1016/j.canlet.2003.11.019 [published Online First: 2004/03/31]

38. Kan Z IK, Hägerstrand I, Chuang VP, Lunderquist A. In vivo microscopy of the liver after injection of Lipiodol into the hepatic artery and portal vein in the rat. *Acta Radiologica* 1989;30(4):419-25.

39. Ohishi H, Uchida H, Yoshimura H, et al. Hepatocellular carcinoma detected by iodized oil. Use of anticancer agents. *Radiology* 1985;154(1):25-9. doi: 10.1148/radiology.154.1.2981114 [published Online First: 1985/01/01]

40. Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Adv Enzyme Regul* 2001;41:189-207. [published Online First: 2001/06/01]

41. Maeda H. Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting. *Proceedings of the Japan Academy – Series B: Physical and Biological Sciences* 2012;88:53-71.

42. Kan Z, Wright K, Wallace S. Ethiodized oil emulsions in hepatic microcirculation: in vivo microscopy in animal models. *Acad Radiol* 1997;4(4):275-82. [published Online First: 1997/04/01]

43. Kritzinger J, Klass D, Ho S, et al. Hepatic embolotherapy in interventional oncology: technology, techniques, and applications. *Clin Radiol* 2013;68(1):1-15. doi: 10.1016/j.crad.2012.06.112 [published Online First: 2012/08/25]

44. Chang JM TW, Pan HB, Yang CF, Lai KH. Transcatheter arterial embolization with or without cisplatin treatment of hepatocellular carcinoma. A randomized controlled study. *Cancer* 1994;74(9):2449-53.

45. Ikeda M, Kudo M, Aikata H, et al. Transarterial chemoembolization with miriplatin vs. epirubicin for unresectable hepatocellular carcinoma: a phase III randomized trial. *J Gastroenterol* 2018;53(2):281-90. doi: 10.1007/s00535-017-1374-6 [published Online First: 2017/08/03]

46. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J Pharm Pharmacol* 2013;65(2):157-70. doi: 10.1111/j.2042-7158.2012.01567.x [published Online First: 2013/01/03]

47. González ID SR, Rodilla EM, Yges EL, Toledano FL. Hypersensitivity reactions to chemotherapy drugs. *Alergol Immunol Clin* 2000;15:161-81.

48. Zheng Z, Pavlidis P, Chua S, et al. An ancestral haplotype defines susceptibility to doxorubicin nephropathy in the laboratory mouse. *J Am Soc Nephrol* 2006;17(7):1796-800. doi: 10.1681/ASN.2005121373 [published Online First: 2006/06/16]

49. Wang Y WY, Tay YC, Harris DC. Progressive adriamycin nephropathy in mice: sequence of histologic and immunohistochemical events. Kidney international. *Kidney international*;58(4):1797-804.

50. Hong K, Khwaja A, Liapi E, et al. New intra-arterial drug delivery system for the treatment of liver cancer: preclinical assessment in a rabbit model of liver cancer. *Clin Cancer Res* 2006;12(8):2563-7. doi: 10.1158/1078-0432.CCR-05-2225 [published Online First: 2006/04/28]

51. Raoul JL, Bourguet P, Bretagne JF, et al. Hepatic artery injection of I-131-labeled lipiodol. Part I. Biodistribution study results in patients with hepatocellular carcinoma and liver metastases. *Radiology* 1988;168(2):541-5. doi: 10.1148/radiology.168.2.2839866 [published Online First: 1988/08/01]

52. E. Towu RA-M, M. Winslet. Uptake of Lipiodol-cytotoxic conjugates by hepatocellular carcinoma cells. *Journal of Pediatric Surgery* 2004;39:203-06.

53. Gaba RC BS, Omene BO, Van Breemen RB, Garcia KD, Larson AC, Omary RA. Ethiodized oil uptake does not predict doxorubicin drug delivery after chemoembolization in VX2 liver tumors. *Journal of Vascular and Interventional Radiology* 2012;23(2):265-73.

54. Arnold RD, Slack JE, Straubinger RM. Quantification of Doxorubicin and metabolites in rat plasma and small volume tissue samples by liquid chromatography/electrospray tandem mass spectroscopy. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004;808(2):141-52. doi: 10.1016/j.jchromb.2004.04.030 [published Online First: 2004/07/21]

55. Rous P, Beard JW. The Progression to Carcinoma of Virus-Induced Rabbit Papillomas (Shope). *J Exp Med* 1935;62(4):523-48. [published Online First: 1935/09/30]

56. Kidd JG, Rous P. A Transplantable Rabbit Carcinoma Originating in a Virus-Induced Papilloma and Containing the Virus in Masked or Altered Form. *J Exp Med* 1940;71(6):813-38. [published Online First: 1940/05/31]

57. Parvinian A CL, Gaba RC. Development, growth, propagation, and angiographic utilization of the rabbit VX2 model of liver cancer: a pictorial primer and "how to" guide. *Diagn Interv Radiol* 2014;20:335-40.

58. Wang D BA, Rhee TK, Woloschak GE, Paunesku T, Salem R, Omary RA, Larson AC. Liver tumors: monitoring embolization in rabbits with VX2 tumors—transcatheter intraarterial first-pass perfusion MR imaging. *Radiology* 2007;245(1):130-9.

59. Ma HL, Xu YF, Qi XR, et al. Superparamagnetic iron oxide nanoparticles stabilized by alginate: pharmacokinetics, tissue distribution, and applications in detecting liver cancers. *Int J Pharm* 2008;354(1-2):217-26. doi: 10.1016/j.ijpharm.2007.11.036 [published Online First: 2008/01/15]

60. Choi YH CJ, Son KR, So YH, Kim W, Yoon CJ, Yoon JH, Chung H, Kim HC, Jae HJ, Kim YI. Novel intraarterial therapy for liver cancer using ethylbromopyruvate dissolved in an iodized oil. *Academic radiology* 2011;18(4):471-8.

61. Zhu AX DD, Sahani DV, Jain RK. HCC and angiogenesis: possible targets and future directions. Nature reviews Clinical oncology. *CLinical oncology* 2011;8(5):292.

62. Yang ZF, Poon RT. Vascular changes in hepatocellular carcinoma. *Anat Rec (Hoboken)* 2008;291(6):721-34. doi: 10.1002/ar.20668 [published Online First: 2008/05/20]

63. Gaba RC, Baumgarten S, Omene BO, et al. Ethiodized oil uptake does not predict doxorubicin drug delivery after chemoembolization in VX2 liver tumors. *J Vasc Interv Radiol*;23(2):265-73.

64. Ramsey DE KL, Soulen MC, Geschwind JF. Chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2002;13:S211-21.

65. Casadaban L, Parvinian A, Gaba RC. Effects of doxorubicin (DOX) delivery on tumor necrosis after drug-eluting bead transarterial chemoembolization (DEB-TACE). *J Vasc Interv Radiol* 2015;26(2):S18.

66. Furuta T, Kanematsu T, Ezaki T, et al. Antitumor effect of lipiodolized doxorubicin on VX2 carcinoma in rabbits. *Jpn J Surg* 1985;15(1):89-91. [published Online First: 1985/01/01]

67. Furuta T, Kanematsu T, Kakizoe S, et al. Selective effect of doxorubicin suspended in lipiodol on VX2 carcinoma in rabbits. *J Surg Oncol* 1988;39(4):229-34. [published Online First: 1988/12/01]

68. Whiteley MS, Patel SB. Modified Tessari Tourbillon technique for making foam sclerotherapy with silicone-free syringes. *Phlebology* 2015;30(9):614-7. doi: 10.1177/0268355514554476
0268355514554476 [pii] [published Online First: 2014/10/08]

69. Varela M, Real MI, Burrel M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007;46(3):474-81. doi: 10.1016/j.jhep.2006.10.020 [published Online First: 2007/01/24]

70. Gaba RC, Yap FY, Martinez EM, et al. Transarterial sorafenib chemoembolization: preliminary study of technical feasibility in a rabbit model. *J Vasc Interv Radiol* 2013;24(5):744-50. doi: 10.1016/j.jvir.2013.01.488 [published Online First: 2013/03/21]

71. Parvinian A, Casadaban LC, Hauck ZZ, et al. Pharmacokinetic study of conventional sorafenib chemoembolization in a rabbit VX2 liver tumor model. *Diagn Interv Radiol* 2015;21(3):235-40. doi: 10.5152/dir.2014.14394 [published Online First: 2015/04/04]

72. Bourget P, Delouis JM. [Review of a technic for the estimation of area under the concentration curve in pharmacokinetic analysis]. *Therapie* 1993;48(1):1-5.

73. Namur J, Citron SJ, Sellers MT, et al. Embolization of hepatocellular carcinoma with drug-eluting beads: doxorubicin tissue concentration and distribution in patient liver explants. *J Hepatol* 2011;55(6):1332-8. doi: 10.1016/j.jhep.2011.03.024 [published Online First: 2011/06/28]

74. Gaba RC, Baumgarten S, Omene BO, et al. Ethiodized oil uptake does not predict doxorubicin drug delivery after chemoembolization in VX2 liver tumors. *J Vasc Interv Radiol* 2012;23(2):265-73. doi: 10.1016/j.jvir.2011.10.022

75. Gao S, Yang Z, Zheng Z, et al. Doxorubicin-eluting bead versus conventional TACE for unresectable hepatocellular carcinoma: a meta-analysis. *Hepatogastroenterology* 2013;60(124):813-20. doi: 10.5754/hge121025 [published Online First: 2013/01/04]

APPENDIX

Appendix A: Tables

Table I: Cohort Characteristics

Measure	Value
Weight at hepatic implantation (Kg)	2.967 ± 0.161
Weight at TACE (Kg)	2.75 ± 0.196
Implant vs. TACE Weight	P= 0.772
Average Tumor Size on Cross Section (cm)	1.2 x 1.24 (± 0.46 x 0.35)
Sex (F:M)	12:0

Table II: Pharmacokinetics Parameters

Measure	Tumor Tissue	Liver Parenchyma	Plasma	P-value
Cmax Doxorubicin (ng/mL)	6,741.5	334.7	1,348.5	
t½ DOX (minutes)	2,106.3 (±188.4)	1,842	149.5(±124.8)	
t½ tumor tissue vs. t½ parenchyma				P=0.062
t½ tumor tissue vs. t½ plasma				P<0.001
AUC[a] (µg min/ml)	14,193	893	16	

[a] area under the concentration versus time curve by the linear trapezoidal method

Appendix B: Figures

Figure 1: Tissue Retention Over Time

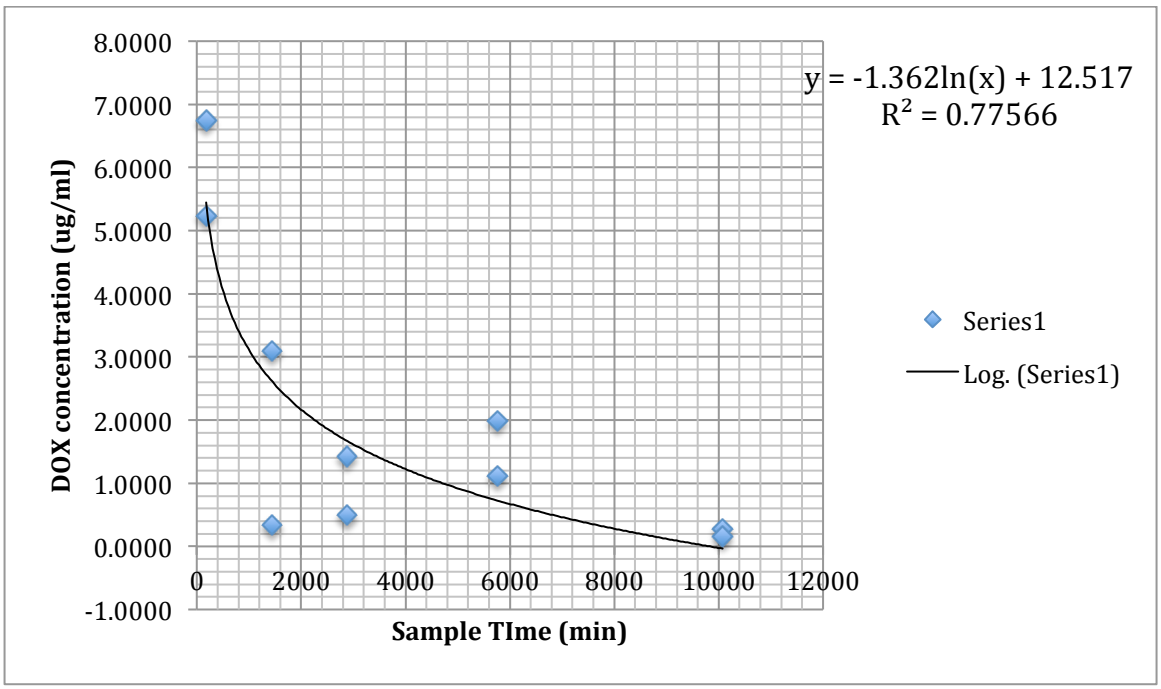
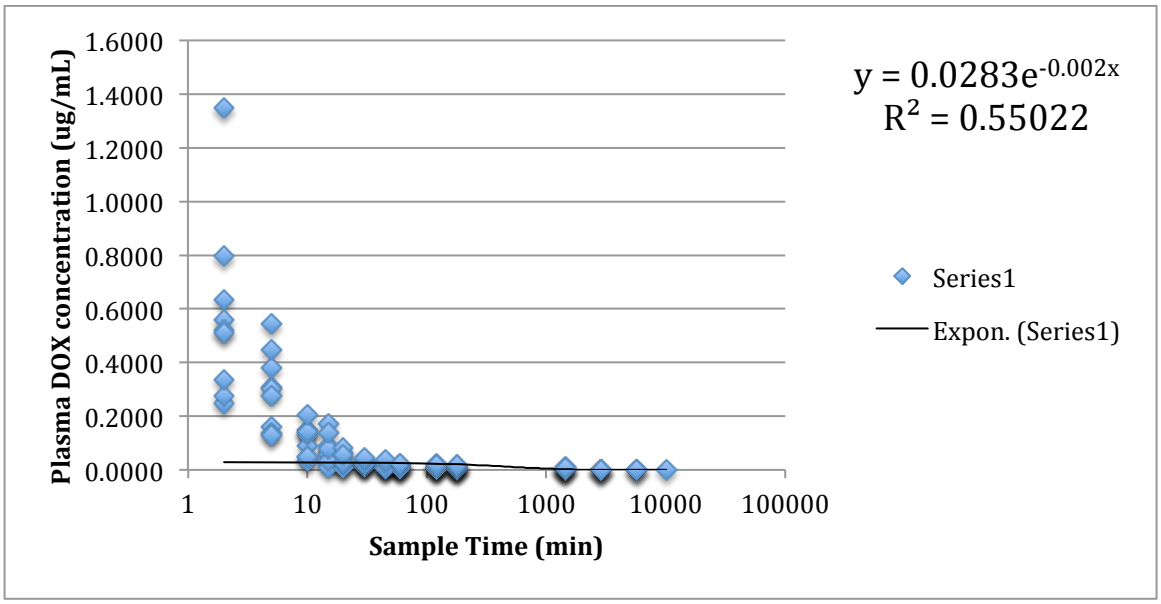


Figure 2: Plasma Concentrations Over Time



VITA

NAME: Joseph Doyle Morrison

EDUCATION: B.S. University of Illinois at Urbana Champaign, 2011

M.A. Loyola University Chicago, 2012

M.D. University of Illinois College of Medicine, 2018

PROFESSIONAL MEMBERSHIP: American Association of Neurological Surgeons

PUBLICATIONS:

Morrison J, Alexander M, Dowd C, Cooke D. Flanking the fenestration: circumferential limb to limb stent assisted coiling of a basilar artery fenestration aneurysm. Journal of Neurointerventional Surgery, accepted 7/17 Manuscript ID neurintsurg-2017-013252.R1

Morrison JD, Mendoza-Elias N, Lipnik AJ, Lokken RP, Bui JT, Ray CE, Gaba RC. Gastric Varices Bleed at Lower Portosystemic Pressure Gradients than Esophageal Varices. J Vasc Interv Radiol

Brown, N. M., Morrison, J., Sporer, S. M. and Paprosky, W. G. The Use of Structural Distal Femoral Allograft for Acetabular Reconstruction of Paprosky Type IIIA Defects at a Mean 21 Years of Follow-Up. Journal of Arthroplasty. 2016. 31(3): 680-683. 10.1016/j.arth.2015.10.020.

Geng, Y., Zhao, Y., Schuster, L.C., Feng, B., Lynn, D.A., Austin, K.M., Stoklosa, J.D., and Morrison, J.D. (2015). A Chemical Biology Study of Human Pluripotent Stem Cells Unveils HSPA8 as a Key Regulator of Pluripotency. Stem Cell Reportshttp://dx.doi.org/10.1016/j.stemcr.2015.09.023

Morrison JD, Lipnik AJ, Gaba RC. TIPS in the treatment of variceal bleeding. Dig Dis Intervent (pending publication manuscript ID: DDI-D-17-00022)

Gaba RC, Mendoza-Elias N, Morrison JD, Valeshabad AK, Lipnik AJ. Decision-making for selection of transarterial locoregional therapy of metastatic neuroendocrine tumors. Semin Intervent Radiol 2017; 34:101-108

Morrison JD, Schlager CK, Lee AE, van Breemen RB, Gaba RC. Does doxorubicin survive thermal ablation? Results of an ex vivo bench top study. J Vasc Interv Radiol 2017; 28:S180

Morrison JD, Mendoza-Elias N, Lipnik AJ, Lokken RP, Bui JT, Ray CE, Gaba RC. Gastric varices bleed at lower portosystemic pressure gradients than esophageal varices. J Vasc Interv Radiol 2017; 28:S166

Mendoza-Elias N, Morrison JD, Lipnik AJ, Lokken RP, Bui JT, Ray CE, Gaba RC. MELD score does not underestimate short-term mortality risk in women versus men after TIPS creation. J Vasc Interv Radiol 2017; 28:S167