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Low Cost Nanoparticle Platform for Diagnosis and Treatment of Colorectal Liver Metastases

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Introduction

Colorectal cancer is the fourth most common cancer worldwide. Approximately 2/3 of patients present with metastasis to the liver, of which 80% of patients would be eligible for some form of liver directed therapy. As such, image guided therapies have developed into a mainstay of the contemporary management of colorectal liver metastatsis (CLM). Currently, therapies include percutaneous ablation strategies (radiofrequency ablation, microwave, cryoablation, irreversible electroporation), trans hepatic arterial therapies (Y90, Drug Eluting Beads), and portal venous intervention (Portal Vein Embolization to induce contralateral liver hypertrophy). However, issues relating to cost (capital equipment), and skill/expertise of hepatic arterial intervention) have resulted in limited patient access.

Recently, it has been discovered that Irinotecan [a key chemotherapy used in colorectal cancer) (CPT-11, Campostar, Pfizer, Canada) is actually a prometabolite, with activation into its active form (SN-38) predominantly within the normal liver parenchyma (not within the tumor). Our research group would like to investigate the possibility of stabilizing this chemotherapeutic through creating a micro/nanoparticle, utilizing inexpensive, off the shelf poppyseed oil extract (ethiodol, Guerbet, Paris, France) as a carrier and imaging agent for either transarterial or transportal administration of targeted therapy. If stabilized emulsions can be created, an entirely new class of delivery, leading to a new type of therapy for liver cancers could added to the therapeutic armamentarium. The intent of this grant application is s to perform the basic bench side testing to confirm the hypothesis that stable nanoparticles can be created, as an initial step towards first in man use. Benefits of this platform would include:

1) Low cost as compared to other liver directed therapy
2) Ease of administration
3) Based on current materials that are readily available in any hospital
4) Novel platform that could scale to other types of low cost nanoparticle methods of manufacture

Objectives

Creation of an inexpensive stable nanoparticle utilizing off the shelf commercially available products.

Hypothesis

Optimized mixing ratios of contrast to lipiodol to chemotherapy will result in the creation of stable nanoparticles that may be used for intraarterial or intraportal payload delivery. Creation of these nanoparticles will usher in a novel, inexpensive way to create therapeutic and diagnostic delivery platforms.

Approach

A three phase approach will be taken to this project, with the objective of working towards first in man trials:

1) Can nanoparticles be created with off the shelf materials, at low cost? If so, can the encapsulation technology be optimized to provide both diagnostic and therapeutic components (theragnostic) on the benchtop?
2) Will administration into the liver (either through a transarterial or transportal route) result in a different pharmacokinetic effect and behavior as compared to infusion of non-nanoparticle chemotherapy in animal models?
3) Can the procedure be translated into a first in human cohort, with optimized imaging and carrier based delivery of chemotherapy nanoparticles?

The first phase of the trial will consist of some preliminary benchtop work at UBC in conjunction with the UBC Department of Pharmaceutical Sciences. Subsequently, dynamic light scattering (DLS) and scanning electron microscopy (SEM) will be conducted in a collaborative effort with the Melancon Lab at MD Anderson (Dr Marites Melancon). Data will then be collected for statistical analysis of stabilization and release patterns with an intent to work towards further grant applications in order to complete animal safety and toxicity studies.

Research Plan

This project is intended to be a basic exploration into the physical properties of off the shelf materials to create stable nanoparticles and determine if these methods would serve as a suitable platform for nanoparticles for both imaging and therapy. If the hypothesis holds true, the methods described would represent the most inexpensive and simplest method to create nanoparticles of any class. The grant application is intended to establish basic costing for disposables and lab time with the intent of further federal (NIH/NSERC/CHIR) levels of support to pursue further research. The findings relating to this preliminary/proof of principle will be intended for oral abstract presentation and publication.

Assuming that stable emulsions can be created in the laboratory, it is the intent of the collaborative group to extend testing (including radiologic visualization and pharmacokinetic activity) into an animal model (likely New Zealand White Rabbits, inoculated with VX2 tumor line into the liver) as an intermediate establishment of safety before proceeding onto first in man study.

The first component of the project is an essential component for any future research. The projected timeline for experimental design execution is one month. The projected time for analysis is two months. No ethics applications will be required at this point however data will be used in conjunction with animal model trials to submit as part of ethics application for first in man.

Role of Applicants

David Liu MD: PI and Interventional Radiologist at University of British Columbia. 2013 CAR Young Investigator Award Recipient. Interests in liver directed therapy and interventional oncology based procedures. (CV Attached)

Alda Tam MD: Associate Professor and Interventional Radiologist at MD Anderson. As a collaborating academic, the concept and methodology has been developed in conjunction with Dr Tam. Dr Tam has specific expertise in nanoparticle creation and also propagating VX2 tumor lines in New Zealand White Rabbit populations. (NIH Biosketch Attached)

Marites Melancon Assistant Professor in the Department of Interventional Radiology at The University of Texas, M.D. Anderson Cancer Center. She received her PhD in Biomedical Science at The University of Texas-Health Science Center at Houston Graduate School of Biomedical Science and joined MD Anderson in the Department of Imaging Physics as an Odyssey Fellow from 2008-2010. She joined Interventional Radiology in 2012 working on the synthesis, characterization and in vivo evaluation of multimodality and multifunctional nanoparticles for image-guided therapy. Her main goal is to apply the "seek and treat" strategy in the development of targeted imaging/therapeutic agents that will eventually be translated to the clinic to improve the management of cancer through early tumor detection and individualized therapy. (NIH Biosketch attached)

Methods/Protocol

To study the change in size of varying amounts of Lipiodol and irinotecan, 12 Lipiodol emulsions containing Visipaque and saline will be mixed using the ratio and concentrations as shown below:

IRI (mg)	Visipaque (mL)	Saline (mL)	Lipiodol (mL)	Total Vol. (mL)	Conc. IRI (mg/mL)	Ratio Dox:Lip
20	4	4	2	10	2.00	10.00
20	4	4	3	11	1.82	6.67
20	4	4	4	12	1.67	5.00
20	4	4	8	16	1.25	2.50
30	4	4	2	10	2.00	10.00
30	4	4	3	11	1.82	6.67
30	4	4	4	12	1.67	5.00
30	4	4	8	16	1.25	2.50
40	4	4	2	10	2.00	10.00
40	4	4	3	11	1.82	6.67
40	4	4	4	12	1.67	5.00
40	4	4	8	16	1.25	2.50

Specifically, irinotecan will be first mixed with saline to dissolve the drug. Visipaque will be added into the drug solution and will be then loaded into a 30 mL syringe. The Lipiodol will be loaded into a separate 30 mL syringe. The two syringes will be then joined by a 3-way stopcock. The Tessari method (20x) will be used to generate emulsion. After the Tessari method, the emulsion will be immediately loaded into a glass cuvette for size measurement using ZetaPals Dynamic Light Scattering instrument (Brookhaven Instruments Corporation). The size will be measured at t= 0, 5, 10, 20, 30, 45, and 60 minutes to monitor the change in particle size over time. Measurements will be performed at room

temperature (25 degrees Celsius). At each time point, 10 measurements will be made. The first three measurements will be then averaged and the standard deviation will be calculated. The pH of the solution will be also measured using a table top pH meter (Mettler Toledo).

The morphology of drug-loaded emulsions will be examined by transmission electron microscopy (TEM). Samples will be placed on 100 mesh formvar coated copper grids treated with poly-L-lysine for 1 hour. Excess samples will be blotted with filter paper, followed by staining with filtered 1% uranyl acetate for 1 min. Stain will be blotted dry from the grids with filter paper and samples will be allowed to dry. TEM will be performed on JEM 1010 transmission electron microscope (JEOL USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images will be collected using the AMT Imaging System (Advanced Microscopy Techniques Corp., Danvers, MA).