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Research paper

Encapsulation and retention of chelated-copper inside hydrophobic nanoparticles: Liquid cored nanoparticles show better retention than a solid core formulation

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ABSTRACT

Motivation: In the field of imaging, ^{18}F-fluorodeoxyglucose (FDG) PET imaging allows evaluation of glucose metabolism and is the most widely used imaging agent clinically for metastatic cancer. While it can certainly detect the metastatic disease, in order to provide a more fully "individualized medicine" strategy of detection and pharmaceutical treatment, what is needed are additional imaging nanoparticles that resemble the subsequently-administered nanoparticle drug delivery system itself. Both of these nanoparticles must also be able to take advantage of what may well be a limited EPR effect in human tumors, which in and of itself still needs to be characterized in the clinic. Administration of FDG, followed by a nanoparticle imaging agent, followed by a therapeutic nanoparticle would constitute such an "individualized medicine strategy", especially for anti-metastasis approaches. It is here that our endogenous-inspired nanoparticle strategies for imaging and therapeutics are focused on encapsulating and retaining imaging ions such as copper inside novel hydrophobic nanoparticles. In this paper, we describe a new approach to label the core of hydrophobic nanoparticles composed of Glyceryl Trioleate (Triolein) with copper using the hydrophobic chelator Octaethyl porphyrin (OEP).

Research plan and methods: The research plan for this study was to (1) *Formulate* nanoparticles and control nanoparticle size using a modification of the solvent injection technique, named *fast ethanol injection*; (2) *Chelate* copper into the octaethyl porphyrin; (3) *Encapsulate* OEP-Cu in nanoparticles: the encapsulation efficiency of copper into liquid nanoparticles (LNP), solid nanoparticles (SNP) and phospholipid liposomes (PL) was evaluated by UV–Vis and atomic absorption spectroscopy; (4) *Retain* the encapsulated OEP-Cu in the liquid or solid cores of the nanoparticles in the presence of a lipid sink.

Results: (1) The size of the nanoparticles was found to be strongly dependent on the Reynolds number and the initial concentration of components for the *fast injection* technique. At high Reynolds number (2181), a minimum value for the particle diameter of ~30 nm was measured. (2) Copper was chelated by OEP in a 1:1 mol ratio with an association constant of $2.57 \times 10^5\ M^{-1}$. (3) The diameter of the nanoparticles was not significantly affected by the presence of OEP or OEP-Cu. The percentage of encapsulation of copper to nanoparticles was >95% at low OEP-Cu concentrations. In the absence of OEP, copper was not detected in nanoparticles demonstrating the role of the hydrophobic chelator OEP in the encapsulation of the otherwise water-soluble copper inside lipid nanoparticles. (4) The *in vitro* retention upon incubation at 37 °C over a 48 h period in the presence of a lipid sink showed a slow transfer of OEP-Cu into the lipid sink ($t_{1/2}$ = 7.7 h) for SNP; for PL there was an almost instantaneous transfer of OEP-Cu into the lipid sink ($t_{1/2}$ = 0.5 h), while for the LNP, all OEP-Cu was retained in the LNP over the full 48 h period.

Conclusions: The main conclusion of this study was that a very hydrophilic ion such as Cu^{2+} can indeed be solubilized and retained in the core of hydrophobic nanoparticles when a hydrophobic molecule (OEP) is used as a chelator. The *fast-injection technique* was shown to provide a very convenient method to formulate both liquid and solid nanoparticles labeled with Cu (well chelated by OEP), with diameters as small as 30 nm, and encapsulation efficiencies higher than 95% when the concentration of OEP-Cu loaded into the nanoparticles was equal to or below 2.5 mol%. This is expected to be sufficient for PET-imaging studies.

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1. Introduction

1.1. Positron Emission Tomography

Positron Emission Tomography (PET) has become the most used qualitative and quantitative technique for cancer imaging in the clinic. It brings several advantages that overcome limitations of optical and other imaging modalities. These include its high signal sensitivity, where, for example, in Immune-PET [1] one-ion-per-antibody can reveal antibody accumulation [2]; the possibility of 3D imaging with good spatial resolution; and the utility of employing the same molecules for *in vivo* preclinical imaging as in clinical imaging [3–8]. Also, as reviewed by Zhu [9], this noninvasive imaging technique provides a functional and metabolic assessment of normal tissue or disease conditions. First administered in humans by Abass Alavi in 1976, ^{18}F-fluorodeoxyglucose PET imaging (FDG-PET) allows evaluation of glucose metabolism in several disorders [10]. It is the most widely used imaging agent clinically for tumor imaging due to the increased glucose metabolism that occurs in most types of tumors. With regard to diagnostics and therapy, in cancer especially, new treatments are focusing on drug delivery by a range of nanoparticles including albumin, gelatin, liposomes, polymers and inorganic NPs [11]. There is an appreciation that, while FDG can certainly detect the metastatic disease, what is needed are additional imaging nanoparticles that resemble the subsequently-administered nanoparticle drug delivery system itself. Administration of FDG, followed by a nanoparticle imaging agent, followed by a therapeutic nanoparticle would constitute an "individualized medicine strategy" of detection and treatment, especially for anti-metastasis approaches [12]. It is here that our endogenous-inspired nanoparticle strategies for imaging and therapeutics are focused on encapsulating and retaining imaging ions such as copper inside novel hydrophobic nanoparticles. The nanoparticles are identical in size and surface properties to therapeutically-active pure drug nanoparticles and are inspired by nature's own nano-delivery system, the LDL. Furthermore, our studies seek to determine which phase of material provides optimal encapsulation and retention, i.e., liquid cored nanoparticles vs a solid core formulation.

1.2. Diagnostic nanomedicine

In the field of diagnostic nanomedicine, a wide variety of nanoparticles labeled with a PET radionuclide have been developed. They include the following: liposomes [13–15] and lipid-coated nanoparticles [16–19], silicon nanoparticles [20,21], polymeric nanoparticles [22–27], iron oxide nanoparticles [28–30], gold nanoparticles [31,32], quantum dots [33] and carbon nanotubes [34]. In preclinical studies, ^{64}Cu is the most used radionuclide to label nanoparticles [13–34]. Typically, nanoparticles are labeled with ^{64}Cu using macrocyclic chelators; 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) being the most used one [23,25,27,31,35]. Despite the wide application of DOTA in labeling nanoparticles with ^{64}Cu, due to their reported low *in vivo* stability (aqueous-ion-exchange), new ^{64}Cu chelators are constantly being developed, mainly based on macrocyclic structures [36,37]. Porphyrins are an example of macrocyclic chelators used recently for the conjugation of ^{64}Cu to nanoparticles, either by using a porphyrin linked to PEG-based block copolymers [38], or by porphyrin-lipids formulated into liposome-like bilayers [39,38] or lipoprotein-like nanoparticles [18]. These lipid-anchored chelators are used for labeling a nanoparticle's surface, and so, again, are subject to aqueous-exchange of the chelated ion. The challenge then is to develop a PET-imageable nanoparticle where the ion is not only well-chelated by a porphyrin, but the porphyrin is trapped inside a hydrophobic environment and not in contact with the aqueous phase. This would have a double benefit of retaining the ion, and, if a hydrophobic porphyrin was used, of encapsulating the chelate in the low dielectric constant, hydrophobic nanoparticle core.

1.3. Optimized Ion chelation for a hydrophobic environment

Based on the chemical ability of the tetra-dentate porphyrin to efficiently chelate cooper [40,41], and the requirement for a low water solubility of porphyrin, we hypothesized that copper ions can be placed in the hydrophobic core of lipid-based nanoparticles when a hydrophobic porphyrin, such as octaethyl porphyrin (OEP) ($S_w \sim 16$ μM, $\log P = 7.8$) is used as the chelator. Also, it has been already proven that porphyrins and porphyrin-derivatives are encapsulated in the core of lipid-based nanoparticles, such as solid-lipid nanoparticles [42] or in reconstituted LDL [43]. However, in those studies, the possibility of chelating a hydrophilic ion to the porphyrin was not evaluated. Placing the copper in the core of nanoparticles, rather than at the particle surface, is expected to have two main advantages: a potentially higher *in vivo* stability due to the protected location of the ion inside the nanoparticle and a higher concentration or radionuclide per nanoparticle. Those advantages have been also stated by other group when ^{64}Cu was encapsulated as a water soluble agent in the inner aqueous core of liposomes [35], suggesting the benefits of placing the radionuclide inside nanoparticles rather than on the particle surface.

1.4. The EPR effect and need for 20–30nm nanoparticles for cancer imaging

The reason for wanting to develop a 20–30 nm nanoparticle for cancer imaging is motivated by the size of the LDL, since this endogenous nanoparticle is known to access tumors and be taken up by cancer cells [44–46]. It has long been known that macromolecules can accumulate in solid tumors through the Enhanced Permeability and Retention (EPR) effect [47]. As described by Maeda in 2000, *"The EPR effect is predominantly observed for biocompatible macromolecules."* The EPR effect continues to be the primary rationale for using nanomedicines (therapeutic nanoparticles) in drug delivery system development for oncology. This is despite the fact that the only data so far obtained in humans are Harrington's 2001 study [48] of just 15 patients using ^{111}In-DTPA-labeled pegylated liposomes, measuring tumor accumulation over 72 h. Also, as reviewed by Kwon and Park et al. [49], the term "*EPR effect*", is often wrongly applied ubiquitously, "*as if all of the iv administered nanoparticles go only to the tumors*". Not surprisingly (and in hind-sight), the few nanotherapeutics that have been approved for the treatment of solid tumors have had a very modest benefit in the patient [50].

One issue that is now becoming clear is that the low therapeutic benefit of some approved nanomedicines in humans differs wildly from the preclinical data in animal models. This critical difference now appears to be attributed to the complexity of the tumor microenvironment for preclinical animal models, compared to the spontaneous tumors that grow in humans [51–53]. The tumors tested in preclinical studies, and many of the subsequent studies that have supported this EPR effect, apparently extending it to nanomedicines, have used implanted subcutaneous tumors in animal models [54]. Studies on tumor accumulation of liposomes have shown that, especially in subcutaneous implanted tumors, there was perivascular extravasation of the 100 nm diameter liposome particles [55]. But not all implanted subcutaneous tumors are the same; there is great variability in vascular permeability. Elegant and comprehensive work by Yuan et al. [56], showed significant