Biomaterials 33 (2012) 2723–2733

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Metal-free and MRI visible theranostic lyotropic liquid crystal nitroxide-based nanoparticles

Benjamin W. Muir [a,**], Durga P. Acharya [a], Danielle F. Kennedy [a], Xavier Mulet [a,b], Richard A. Evans [a], Suzanne M. Pereira [a], Kim L. Wark [a], Ben J. Boyd [b], Tri-Hung Nguyen [b], Tracey M. Hinton [c], Lynne J. Waddington [a], Nigel Kirby [d], David K. Wright [e], Hong X. Wang [e], Gary F. Egan [e], Bradford A. Moffat [f,*]

[a] CSIRO Materials Science and Engineering, Bayview Avenue, Clayton 3168, Australia
[b] Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville 3052, Australia
[c] CSIRO Livestock Industries, Australian Animal Health Laboratory, East Geelong 3219, Australia
[d] Australian Synchrotron, Clayton 3168, Australia
[e] Howard Florey Institute, The University of Melbourne, 3010, Australia
[f] The University of Melbourne, Department of Radiology, Parkville 3050, Australia

ARTICLE INFO

Article history:
Received 18 November 2011
Accepted 6 December 2011
Available online 29 December 2011

Keywords:
Cubosome
Contrast agent
Lyotropic liquid crystal
Cytotoxicity
Maximum tolerated dose
High-throughput

ABSTRACT

The development of improved, low toxicity, clinically viable nanomaterials that provide MRI contrast have tremendous potential to form the basis of translatable theranostic agents. Herein we describe a class of MRI visible materials based on lyotropic liquid crystal nanoparticles loaded with a paramagnetic nitroxide lipid. These readily synthesized nanoparticles achieved enhanced proton-relaxivities on the order of clinically used gadolinium complexes such as Omniscan™ without the use of heavy metal coordination complexes. Their low toxicity, high water solubility and colloidal stability in buffer resulted in them being well tolerated *in vitro* and *in vivo*. The nanoparticles were initially screened *in vitro* for cytotoxicity and subsequently a defined concentration range was tested in rats to determine the maximum tolerated dose. Pharmacokinetic profiles of the candidate nanoparticles were established *in vivo* on IV administration to rats. The lyotropic liquid crystal nanoparticles were proven to be effective liver MRI contrast agents. We have demonstrated the effective *in vivo* performance of a T1 enhancing, biocompatible, colloidally stable, amphiphilic MRI contrast agent that does not contain a metal.

1. Introduction

The development of various nanoparticle formulations as potential MRI contrast agents is an area of ever growing research. The benefits of developing nanoparticle formulations include the potential to target these agents to diseased tissues such as tumors. Previously this has been achieved passively via the enhanced permeability and retention effect (EPR) [1–3] or actively via attachment of targeting ligands such as folate [4], peptides [5] or antibodies [6]. In addition, the use of nanoparticles may enable multimodal theranostic agents to be developed for both imaging diseased tissue, and treating the disease by controlled release of drugs [7,8]. Few nanoparticle formulations have been clinically approved due to the immense physico-chemical, biological and regulatory hurdles that confront the nanotechnologist when devising successful nanoparticle and coating formulations [8].

The main aim of scientists developing new and improved MRI theranostic materials is to produce nanoparticles which provide strong signal contrast while maintaining low toxicity and ease of intra-venous delivery (small volume bolus, stability in saline, increased blood half life and low viscosity). Contrast agents are used clinically when poor contrast of the diseased tissue is observed during MRI scanning (as seen commonly when imaging brain and liver lesions). Signal enhancement is achieved by contrast agents that decrease the water spin-lattice relaxation time (T1) during the acquisition of T1 weighted MR images, while signal suppression is achieved by agents that decrease the water spin-spin relaxation time (T2) during the acquisition of T2 weighted MR images. The contrast agent relaxivity (r1 or r2) is a measure of the relative effectiveness of a given contrast agent and has units of $s^{-1}mM^{-1}$. To date most nanoparticle MRI contrast agent formulations (whether T1 or T2

* Corresponding author. Fax: +61 393428369.
** Corresponding author. Fax: +61 395452515.
E-mail addresses: ben.muir@csiro.au (B.W. Muir), brad.moffat@rmh.org.au (B. A. Moffat).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.12.018

enhancing) have been used as liver contrast agents due primarily to uptake by the reticuloendothelial system (RES) after bolus delivery [8,9]. Most clinically used T1 enhancing agents contain gadolinium (Gd) [10,11] which is highly toxic as a free trivalent ion [12]. Although the Gd metal in clinically used contrast agents are chelated [10] the metal may still potentially leach. Certain agents may cause adverse reactions in patients with renal failure resulting in a debilitating disease called nephrogenic systemic fibrosis [13]. As a result, phasing out the use of heavy metal based contrast agents and finding suitable alternatives is of great interest to the field. To date, non-heavy metal based materials investigated as T1 enhancing agents have essentially been limited to ^{19}F [14,15] and nitroxide enriched compounds [16–18].

One of the greatest challenges to the materials scientist in the preparation of suitable MRI contrast agent nanoparticles is the production of highly stable colloidal dispersions that have negligible cytotoxic properties [8]. To this end we have developed the use of lyotropic liquid crystal nanoparticles that contain a paramagnetic nitroxide lipid to provide T1 contrast rather than the conventionally used gadolinium based compounds. This may allow the field to move away from the toxicity issues associated with gadolinium based contrast agents. Previous reports have shown the applicability of using gadolinium functionalized lipids incorporated into lyotropic mesophase liquid crystal nanoparticles as potential MRI agents *in vitro* [19,20]. The use of amphiphilic lipids such as phytantriol and glyceryl monooleate (GMO) (structure in Fig. 1) result in the formation of distinct lyotropic mesophases of varying complexity and dimensionality. Glycerol monooleate contains an ester group that is susceptible to hydrolysis and therefore biodegradation *in vivo*. The common phases seen when using lyotropic liquid crystal materials include lamellar phase (L) (1-D) comprised of stacked bilayer sheets, the hexagonal phase (H2) (2-D) which can be conceptualized as infinitely long hexagonally packed rods with an aqueous interior and finally the cubic phase (Q2) (3-D) consisting of a bi-continuous network of hydrophilic and hydrophobic domains containing two continuous water channels. The Q2 phase represents a family of closely related structures, where the underlying crystal lattice can be described by the gyroid (G), diamond (D) and primitive (P) minimal surfaces, which correspond to the *Ia3d* (G), *Pn3m* (D) and *Im3m* (P) crystallographic space groups, respectively. The 3-dimensional structure affords a self-assembled scaffold with a remarkably high surface area and extensive porosity. These properties, coupled with the liquid crystalline nature of the phase, result in a structure that was found to not be susceptible to osmotic or mechanical rupture in contrast to the properties of liposomes [21] or micelles. The thermodynamic stability of the Q2 phase affords a structure that co-exists in equilibrium with excess water over a broad temperature range. The dispersion of the bulk gel-like cubic phases can be achieved by mechanical or ultrasonic treatment and results in the formation of nanometer-sized particles that retain the internal cubic structure of the parent bulk cubic phase. The incorporation of additives to such materials may result in the formation of other phases such as the inverse hexagonal phase (H2) due to an alteration in the spontaneous curvature of the lyotropic liquid crystal assemblies. Dispersions of hexagonal phase nanoparticles are commonly called hexosomes and cubic phase nanoparticles are referred to as cubosomes™ [22–26].

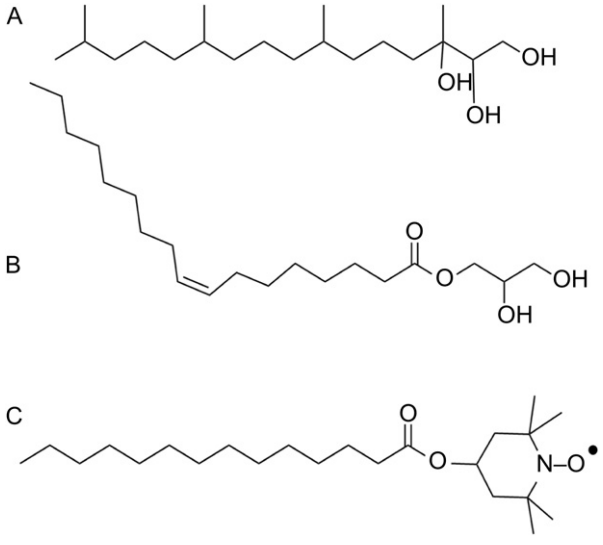

Fig. 1. Chemical structure of phytantriol amphiphile (top structure), glyceryl monooleate amphiphile (middle structure, the main component of Myverol) and the nitroxide lipid (bottom structure) used to make lyotropic liquid crystal, MRI contrast agent nanoparticles.

The aim of this study was to investigate the benefit of incorporating a myristic nitroxide lipid (structure in Fig. 1) into lyotropic liquid crystal nanoparticles. Nitroxides are stable, organic free radicals with an unpaired (paramagnetic) electron and are therefore capable of shortening the MRI relaxation times [18]. Once inside the body, an equilibrium exists between the paramagnetic (contrast enhancing) nitroxide and the reduced non-paramagnetic (non-contrast enhancing) hydroxylamine [27]. Previous studies have shown that these compounds can be useful for imaging intracellular redox metabolism by MRI [28,29], because the ratio of the two states was dictated by the local oxygen and redox environment. In addition these compounds have been shown to have potential for controlling hypertension and weight, preventing damage from reperfusion injury, and treating neurodegenerative diseases and ocular damage [29,30]. It was hypothesized that encapsulating the nitroxide lipid inside the lyotropic mesophase liquid crystal nanoparticles would extend the nitroxide radicals half-life *in vivo* making it an effective MRI contrast agent with an acceptable cytotoxicity profile. Furthermore, the presence of confined water channels in cubic and hexagonal phase nanoparticles, and their greater surface area compared to liposomes may result in enhanced relaxivities of the nitroxide lipid due to rotational correlation constant and proton exchange processes.

To investigate these hypotheses, nanoparticles were synthesized using two different bulk cubic phase forming lipids. In this study the effect of nanoparticle structure on relaxivity, cytotoxicity, maximum tolerated dose in rats and efficacy of the contrast agents *in vivo* for nitroxide loaded cubosomes and hexosomes, was investigated. The cubic and hexagonal phase nanoparticles have a viscosity approximately equal to water, which makes it desirable for bolus delivery of MRI contrast agents. Previous formulations of these types of nanoparticles have shown them to have high colloidal stability and low cytotoxicity through the appropriate selection of the amphiphile used to form the lyotropic liquid crystal phase [31].

2. Materials and methods

2.1. Self-assembly of the lyotropic liquid crystal nitroxide containing nanoparticles

Two bulk cubic phase forming lipids were used in this work, phytantriol (DSM Nutritional Products, GmbH) and Myverol (Bronson & Jacobs, Sydney) which were used as received. Myverol™ was used as a commercially available source of GMO which was the main lipid component of this product. Samples for screening the T1 and T2 relaxivities were prepared in a high-throughput manner using a Chemspeed Accelerator™ SLTII robotic synthesis platform equipped with a 4-needle head and probe sonicator tools. As previously reported [32], stock solutions of the phytantriol, Myverol and nitroxide lipid were prepared in a poly(ethylene) 96 well plate (2 mL capacity per well) containing chloroform, 0.5 mg of bulk lipid and an appropriate