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Comparison of pharmaceutical nanoformulations for curcumin: Enhancement of aqueous solubility and carrier retention

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ABSTRACT

Curcumin, originally used in traditional medicine and as a spice, is one of the most studied and most popular natural products of the past decade. It has been described to be an effective anti-inflammatory and anti-cancer drug and protects against chronic diseases such as rheumatoid arthritis and atherosclerosis. Despite these promising pharmacological properties, curcumin is also very lipophilic, which makes its formulation challenging. Ideally the nanocarrier should additionally also retain the encapsulated curcumin to provide target tissue accumulation.

In this study we aimed to tackle this aqueous solubility and carrier retention challenge of curcumin by encapsulating curcumin in different nanoparticles. We successfully loaded LDL (30 nm), polymeric micelles (80 nm), liposomes (180 nm) and Intralipid (280 nm) with curcumin. The relative loading capacity was inversely related to the size of the particle. The stability for all formulations was determined in fetal bovine serum over a course of 24 h.

Although all curcumin-nanoparticles were stable in buffer solution, all leaked more than 70% of curcumin under physiological conditions. Altogether, tested nanoparticles do solve the aqueous insolubility problem of curcumin, however, because of their leaky nature, the challenge of carrier retention remains.

1. Introduction

1.1. Curcumin

Curcumin is isolated from *Curcuma longa* and used as a spice and in traditional medicine in Asia (Naksuriya et al., 2014) and is together with quercetin and resveratrol among the most studied natural products. It has very potent pharmacological capabilities, exhibiting pleiotropic effects including the inhibition of TNF-α, IL-1β, IL-12, INF-γ, EGF, HGF, Stat3 and NF-κβ (Naksuriya et al., 2014). Furthermore, curcumin has been described to have anti-cancer, antiviral, anti-fungal, antioxidant, anti-angiogenic and anti-inflammatory properties (Flora et al., 2013) and shows activity in chronic diseases such as diabetes type II, rheumatoid arthritis, multiple sclerosis, Alzheimers' disease and atherosclerosis (Naksuriya et al., 2014).

Despite the promising anti-inflammatory effects shown in an extensive collection of papers, there are some critical arguments concerning the real pharmacological actions of curcumin. Curcumin is one of the compounds that in drug screening assays easily gives positive hits, caused by its chemical structure rather than real pharmacological actions (Baell, 2015; Baell and Walters, 2014). One of the reasons why curcumin is such a highly active compound and acts like a drug in most assays is the fact that it alters membrane properties. There is usually little evidence that curcumin has a direct interaction with a protein (Ingólfsson et al., 2014), which would make it a *bona fide* drug. Nevertheless, the non-specific membrane perturbations seem to be beneficial in a variety of – inflammation associated – conditions *in vivo*, making curcumin an interesting pharmaceutical compound.

Of the physicochemical properties of curcumin (Fig. 1) with a molecular weight (MW) of 368.39, lipophilicity (log*D* at 7.4) of 4.12 and a topological polar surface area (tPSA) of 93.06 Å, especially the low aqueous solubility stands out (Lipinski et al., 2001; Veber et al., 2002). Furthermore, in biological systems,

Abbreviations: cur-intra, curcumin loaded intralipid; cur-LDL, curcumin loaded LDL; cur-lip, curcumin loaded liposomes; cur-mic, curcumin loaded polymeric micelles; EPR, enhanced permeability and retention; MW, molecular weight; tPSA, topological polar surface area.

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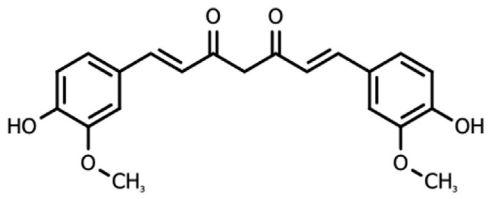

Fig. 1. Molecular structure of curcumin.

curcumin is an unstable molecule, with rapid metabolism and degradation, for example into vanillin, and quick systemic elimination *in vivo* (Flora et al., 2013; Wang et al., 1997). Additionally, curcumin has poor uptake after oral administration with about 75% direct elimination *via* the feces (Wang et al., 1997).

Altogether these highly unfavorable characteristics of curcumin ask for a solution to improve the bioavailability and insolubility. A potential solution are nanomedicine formulations. Indeed, curcumin has been encapsulated in a wide selection of nanocarriers to improve its solubility, but mainly to increase its bioavailability and protection against systemic degradation (Flora et al., 2013; Mohanty et al., 2012; Naksuriya et al., 2014). Ideally, curcumin would also be retained in the carrier to take advantage of the carrier's ability to target sites of disease.

1.2. Nanoparticle selection

There is a variety of nanoparticles available that could potentially improve delivery and bioavailability of curcumin. Nanoparticles which are between 10 and 100 nm have the ability to take advantage of the enhanced permeability and retention (EPR) effect (Petros et al., 2010). The EPR effect is the phenomenon of enhanced vascular endothelial permeability and impaired lymphatic drainage at inflammatory sites (Matsumura and Maeda, 1986). Nanoparticles passively diffuse through the endothelial lining and stay at the inflammatory site, because of poor lymphatic drainage, where they release their contents.

Often used, best known and FDA approved nanoparticles are the liposomes. These nanoparticles are typically 100–150 nm and consist of a phospholipid bilayer which can vary in composition, surrounding an aqueous core. Liposomes are suitable for hydrophilic compounds, which can reside in the core of the particle. Lipophilic compounds can nest in the fatty environment of the lipid bilayer, however, because of the dynamic nature of lipid exchange, drugs can easily be extracted. Liposomes are often the first of choice because of their ease of formulation, lipid composition tuning possibilities and the extensive experience with the system (Allen and Cullis, 2013; Petros et al., 2010).

Micelles are made from a single layer of amphiphilic molecules, such as phospholipids or polymers, surrounding a lipophilic core. These nanoparticles are particularly convenient for lipophilic pharmaceuticals because of their relatively high relative hydrophobic volume. An example of a micelle, however not often employed for drug delivery, is Intralipid. This micelle formulation is given as an intravenous nutrient. It is around 270 nm in diameter, readily available, cheap and FDA approved (Fresenius Kabi LTD, 2015). Therefore it is an undemanding starting point for delivery of lipophilic drugs.

Natural micelles which can be exploited as drug carriers are high density lipoprotein (HDL) and low density lipoprotein (LDL). The first one is a small (~10 nm) micelle consisting of a single lysophospholipid layer and a lipophilic core. The particle is stabilized by the lipoprotein ApoA1. A mimic of this nanoparticle can be synthesized in the lab, as ApoA1 easily attaches itself to the phospholipid layer. This nanoparticle is successfully used as drug carrier (Duivenvoorden et al., 2014) and as contrast agent in medical imaging (Cormode et al., 2009). LDL is slightly larger than HDL (~25 nm) and also has a phospholipid monolayer with cholesterol and a lipophilic core containing cholesteryl esters and triglyceride (McNamara et al., 1996). The lipoprotein ApoB100 which is wrapped around the molecule is one of the largest known monomeric proteins (Segrest et al., 2001) and is not able to be exchanged, as is the case for ApoA1. This makes the particle highly stable, and impossible to mimic in the lab. LDL has been used as a drug carrier though, by exchanging the core for a drug of choice using a core extraction method (Krieger, 1986; Zheng et al., 2002) or sonication (Allijn et al., 2013).

Polymeric micelles are especially designed for drug delivery and usually have a diameter below 100 nm. Like liposomes, these micelles can be built from a variety of polymers and have been investigated extensively (Petros et al., 2010; Shi et al., 2013). Often, these micelles suffer from instability in biological environments, due to exchange of the amphiphilic molecules with other surfaces. A solution to this exchange, is the covalent coupling of the micellar matrix within the core (Rijcken et al., 2007). Still, these core-crosslinked micelles suffer from rapid release of the lipophilic payload which can be circumvented by also covalently linking these molecules to the covalently linked micelle matrix (Talelli et al., 2010). A recent advancement in micelle formulations circumventing the need for covalent crosslinking are the $\pi\pi$-stacking micelles (Shi et al., 2013). These micelles derive their stability from the interactions between the π-orbitals of stacked aromatic rings. Since curcumin contains two aromatic rings it appears a suitable molecule to be employed in this strategy.

In this study, we compare four nanoparticles, namely liposomes, Intralipid, polymeric micelles and LDL as potential nanocarriers for their ability to enhance the aqueous solubility of curcumin and to retain the drug in the presence of plasma (Fig. 2).

2. Materials and methods

2.1. Chemicals

1,2-Dipalmitoyl-sn-*glycero*-3-phosphocholine (DPPC) and 1,2-Distearoyl-sn-*glycero*-3-phosphoethanolamine (DSPE-PEG_{2000}) and 1,2-distearoyl-sn-*glycero*-3-phosphocholine (DSPC) were purchased from Lipoid GmbH, Germany. Cholesterol, methanol UPLC grade, tetrahydrofuran (THF) and triethylamine (TEA) were acquired from Sigma-Aldrich Chemie GmbH, Germany. Fetal bovine serum (FBS) was purchased from Lonza, Belgium, curcumin from Chengdu Biopurity Phytochemicals Ltd

Ethanol absolute from Merck KGaA, Germany, HEPES from Acros Organics, Belgium, Intralipid from Fresenius Kabi Nederland BV, 1-Myristoyl-sn-*glycero*-3-phosphocholine (MHPC) was purchased from Bachem, Germany and NaCl from Fisher Chemical, Fisher Scientific GmbH, Germany. MilliQ water was obtained from a Merck Millipore Q-POD. mPEG-HPMA-Bz polymer was a kind gift from Aida Varela Moreira of Utrecht University.

2.2. Curcumin property prediction and structure evaluation

The molecular structure of curcumin was identified in SciFinder (Chemical Abstract Service (CAS) (Chemical Abstract Service, 2013)) and physicochemical properties were calculated by ChemAxon's Instant JChem (ChemAxon, 2015).

2.3. Formulation of curcumin-liposomes

The liposomes were assembled with DPPC, DSPE-PEG_{2000} and cholesterol in a molar ratio of 1:0.08:0.52. Lipids were placed in