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Microwave-assisted formulation of solid lipid nanoparticles loaded with non-steroidal anti-inflammatory drugs

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ABSTRACT

Stearic acid-based solid lipid nanoparticles (SLNs) were prepared using the microwave assisted one-pot microemulsions procedure pioneered by our group. In this study, non-steroidal anti-inflammatory drugs (NSAIDs) including indomethacin, ketoprofen and nimesulide were selected as ideal "test" drugs, based on their poor water solubility. The model drugs were incorporated within the SLNs by the microwave-assisted procedure at the time of SLN production. The microwave-produced drug-loaded SLNs were evaluated in terms of their physicochemical characteristics, drug release behavior and their uptake into against A549 cell line (human lung epithelial cells). The microwave-produced drug-loaded SLNs had a small particle size distribution, negative zeta potential and high encapsulation efficiency. The drug release studies were consistent with a core-shell structure of SLNs (probably a drug-loaded shell) which results in biphasic drug release from the SLNs. The drug release kinetics suggested a good fit of the release data to the Makoid-Banakar model and was governed by Fickian diffusion. The drug-loaded SLNs showed concentration-dependent cytotoxicity and reduced IL-6 and IL-8 secretion in lipopolysaccharide-induced cells. All of the above findings suggest that the microwave-produced SLNs could be promising drug carriers of NSAIDs and will further facilitate their development for topical, oral and/or nasal administration.

1. Introduction

A large number of potent but poorly water soluble drug candidates have been identified in the past few decades due to the success of emerging technologies including high-throughput screening and combinatorial chemistry. The low water solubility of these drug candidates results in diminished efficacy. One such group of drugs are the non-steroidal anti-inflammatory drugs (NSAIDs).

The use of non-steroidal anti-inflammatory drugs (NSAIDs) has increased considerably over the past two decades. These are the most commonly prescribed preparations, available both as prescription and over-the-counter medications. They have prominent analgesic, antipyretic and anti-inflammatory properties (Day and Graham, 2013) and are frequently used in the treatment of inflammatory disorders such as inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, low back pain and other joint diseases (Okyar et al., 2012). Despite their wide application, poor water solubility of these drugs limits their therapeutic use.

Encapsulation of NSAIDs in drug vehicles is one of the few approaches that have been investigated for overcoming the aforementioned disadvantages. The delivery vehicle (encapsulating agent) has the potential to protect the drug from absorptive loss and/or destruction while, for example, it passes through the digestive system and may even allow targeting of drug release at specific sites in the body. This allows for higher efficacy without using more drug. NSAIDs have been successfully encapsulated into polymeric particles (Del Gaudio et al., 2009; Kluge et al., 2009; Arida and Al-Tabakha, 2007; Graves et al., 2008), liposomes (Maestrelli et al., 2005, 2006; Srinath et al., 2000), dendrimers (Murugan et al., 2014) and microemulsions (Rhee et al., 2001). Although there has been great success in encapsulating NSAIDs, each of the drug carriers so far used still suffers from some limitations such as the use of toxicologically harmful reactive cross-linkers and carcinogenic monomers, accumulation of polymers due to its slow degradation, the possible production of toxic metabolites, increased mobility, reduced stability and drug leakage. These studies (Del Gaudio et al., 2009; Kluge et al.,

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2009; Arida and Al-Tabakha, 2007; Graves et al., 2008; Maestrelli et al., 2005, 2006; Srinath et al., 2000; Murugan et al., 2014; Rhee et al., 2001) suggest that NSAIDs are well accepted in encapsulation and their drug delivery is well characterized. The selection of NSAIDs in encapsulation studies for solid lipid nanoparticles (SLNs), the drug carriers of interest in this study, seems appropriate. SLNs may overcome many of those disadvantages, without compromising the advantages of encapsulation.

SLNs are colloidal carriers prepared from biodegradable and biocompatible lipids that have generally-recognized-as-safe (GRAS) status (Gastaldi et al., 2014). The lipids used in the preparation of SLNs are solid at room and body temperature. The high melting lipid forms the solid core of the SLN which is coated with nontoxic surfactants that stabilize the SLNs in dispersions (Dan, 2014). SLNs have been actively sought as alternative drug carriers because of their unique properties including biocompatibility, tolerability, physical stability, feasibility of large scale production, possibility of sustained-release or controlled-release of drugs, possibility of specific site targeting and improved bioavailability of drugs (Shah et al., 2015a).

In our previous studies, we reported a novel microwave-assisted microemulsion process to produce SLNs (Shah et al., 2014a, 2016a,b,c). This study indicated that the microwave-assisted procedure produced SLNs with improved physicochemical characteristics over conventional SLNs. The application of microwave-produced SLNs is quite innovative and remains largely unexplored. In the current study, we are interested in applying the recently reported microwave-assisted microemulsion method to prepare SLNs as an alternative delivery system for NSAIDs. We have selected indomethacin, ketoprofen and nimesulide (Fig. 1) as model drugs based on their physicochemical properties (Table 1). The selected model drugs have been used in the treatment of rheumatoid arthritis, gout, synovitis, osteoarthritis and other inflammatory conditions. We evaluated the physicochemical characteristics, drug release kinetics and *in vitro* behavior of microwave-produced drug-loaded SLNs. Specifically, cytotoxicity against A549 cells, the ability to breach the cellular membrane prior to release of drug encapsulated within the SLNs and their anti-inflammatory effects on lipopolysaccharide (LPS)-induced cells were investigated.

2. Experimental section

2.1. Materials

Stearic acid (solid lipid) and Tween® 20 (surfactant) were purchased from ICN Biomedicals Inc. (USA) and Merck (Australia) respectively. Rhodamine 123 was purchased from Sigma-Aldrich (Australia). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 were purchased from Sigma-Aldrich (Australia). Dimethyl sulfoxide (DMSO) was obtained from Merck (Australia). 4′, 6-diamidino-2-phenylindole, dihydrochloride (DAPI) and CellMask™ Deep Red Plasma membrane stain were obtained from Molecular probes (USA). Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Invitrogen Corp.), fetal bovine serum (FBS), penicillin G sodium (10,000 units/mL) and streptomycin (10,000 μg/mL) were obtained from Invitrogen Technologies (Australia). Dulbecco's phosphate buffered solution (PBS) was provided by Sigma-Aldrich (Australia). Ultra-purified water was obtained from a MilliQ® Plus purification system (Millipore, Germany) and all other chemicals and reagents were commercially available and of analytical grade.

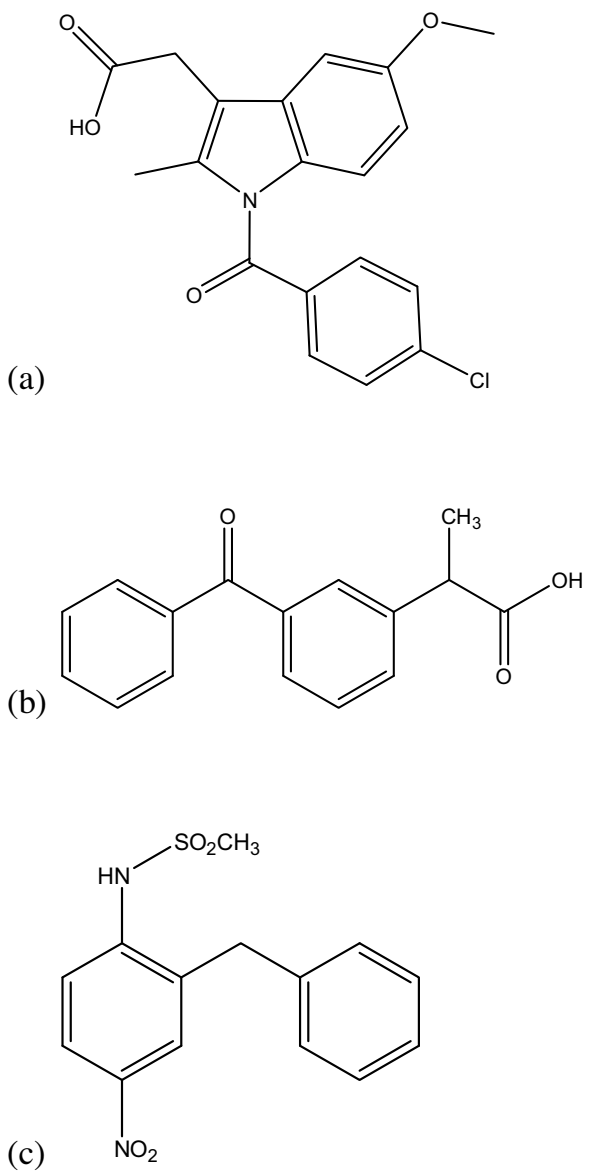

Fig. 1. Chemical structures of non-steroidal anti-inflammatory drugs used in this study. (a) Indomethacin, (b) Ketoprofen and (c) Nimesulide.

2.2. Methods

2.2.1. Preparation of solid lipid nanoparticles

The SLNs were prepared by the novel microwave-assisted microemulsion technique as described previously by Shah et al. (2014a). Briefly, a hot o/w microemulsion comprising of stearic acid (150 mg), Tween® 20 (150 μL) and water (1.35 mL) was prepared by the one-pot microemulsion procedure in a microwave reactor tube with constant stirring at 80 °C with a variable microwave power not exceeding 18 W for 10 min using a 2.45 GHz Discover LabMate microwave synthesizer (CEM Corp., USA). For drug-loaded SLNs, the drug (5 mg) was added to the microwave reactor tube prior to microemulsion synthesis. Upon completion of the microemulsion synthesis, the hot o/w microemulsion was dispersed immediately into cold water (2–4 °C) under constant magnetic stirring to generate SLN dispersions. Rhodamine-loaded SLNs prepared in our previous study (Shah et al., 2016a) were used to evaluate the cellular uptake of SLNs. Rhodamine 123 (0.03%