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Self-assembling gelling formulation based on a crystalline-phase liquid as a non-viral vector for siRNA delivery

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ABSTRACT

Liquid crystalline systems (LCSs) form interesting drug delivery systems. These include *in situ* gelling delivery systems, which present several advantages for use as self-assembling systems for local drug delivery. The aim of this study was to develop and characterize *in situ* gelling delivery systems for local siRNA delivery. The influence of the components that form the systems was investigated, and the systems were characterized by polarized light microscopy, Small Angle X-ray Scattering (SAXS), swelling studies, assays of their ability to form a complex with genes and of the stability of the genes in the system, as well as assays of *in situ* gelling formation and local toxicity using an animal model. The system containing a mixture of monoglycerides (MO), oleylamine (OAM), propylene glycol (PG) and tris buffer (8.16:0.34:76.5:15, w/w/w/w) was considered the most appropriate for local siRNA delivery purposes. The molecular structure was characterized as hexagonal phase; the swelling studies followed a second order kinetic model and the water absorption was a fast process reaching equilibrium at 2 h. The system formed a complex with siRNA and remained in a stable form. The gel was formed *in vivo* after subcutaneous administration of a precursor fluid formulation in mice and was biodegradable in 30 days. The inflammatory process that took place was considered normal. Therefore, the developed liquid crystalline delivery system shows the appropriate characteristics for use as a local siRNA delivery method for gene therapy.

1. Introduction

Liquid crystals, defined as the state of matter whose symmetric and mechanical properties are intermediate between a crystalline solid and an isotropic liquid (Singh, 2000), have been widely used in the pharmaceutical area due to their ability to form delivery systems, such as nanodispersions and *in situ* gelling systems. These vehicles appear to be potential delivery system for drugs, peptides and genes because they have the ability to incorporate compounds with different solubility, protect them from enzymatic and physical degradation, increase skin penetration and control drug release (Lopes et al., 2006b, 2006a, 2007; Vicentini et al., 2013b).

Liquid crystalline systems (LCSs) are formed from unsaturated monoglycerides (MO), such as monoolein and monolinolein. The ability of the MO to absorb water and to form a viscous liquid crystalline phase, such as a hexagonal or a cubic phase, is well described in the literature and enables the formation of a gel *in situ* (Chang and Bodmeier, 1997c, 1998; Lara et al., 2005; Rizwan et al., 2009). *In situ* gelling delivery systems present several advantages, such as easy administration, simple production, minimal invasiveness (i.e., less painful administration), dose reduction capability and local, sustained delivery (Hatefi and Amsden, 2002).

The importance of *in situ* gelling delivery systems has increased lately because many new macromolecules, such as proteins and nucleic acids, that are easily degraded when administered by conventional routes, have been discovered as a consequence of the development of molecular biology studies and genomic information. These new macromolecules require the development of delivery systems that can protect them and release them in a localized and sustained way (Agarwal and Rupenthal, 2013; Hatefi and Amsden, 2002).

Among the new therapeutic discoveries, gene therapy is promising because it provides a unique way to regulate, repair, replace,

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add, delete or silence a genetic sequence that is identified as beneficial or harmful to the appropriate functioning of the body (Wirth et al., 2013). Thus, gene therapy has the potential to deeply change the way to address and treat both inherited disorders and acquired diseases (Nabel, 2004). In this context, the approach of silencing genes has drawn the attention of many researchers. It can be achieved by different processes. These include RNA interference (RNAi), which inhibits endogenous genes by the degradation of the messenger RNA using corresponding double-stranded RNA. Small interfering RNA (siRNA) is a short double-stranded molecule that can silence the expression of particular proteins through this RNAi process. Compared with other antisense/gene therapies, siRNA presents several advantages, including straightforward synthesis, specificity, potency and robustness. Its site of action is in the cytoplasm and so it presents a smaller risk of promoting toxic effects (Vicentini et al., 2013a).

Although significant progress has been made over the past years, many challenges for effective siRNA therapy must be overcome, including delivery to a specific cell population, efficient gene transfection and avoiding the immune response (Nabel, 2004). siRNA delivery systems have neutralized the negatively charged phosphate backbone of nucleic acids to avoid charge repulsion against the anionic cell membrane; they have also condensed the bulky structure of DNA to appropriate length scales for cellular internalization and protected the nucleic acid from nuclease degradation (Reischl and Zimmer, 2009; Vicentini et al., 2013a). Both viral and non-viral delivery systems have been developed to overcome these problems, showing success in delivering the siRNA to the intracellular environment and inducing RNA interference (RNAi). Non-viral delivery systems, commonly consisting of siRNA incorporated into lipids and polymers, are promising siRNA delivery systems because they are safer than viral vectors; however, efforts should be made to improve the mediation of gene expression by these vectors (Reischl and Zimmer, 2009). One factor that interferes with gene transfer is the administration route. Local delivery of siRNA using non-viral vectors is advantageous because it provides higher bioavailability, efficient targeting of the affected cells and reduced side effects in addition to enabling the use of lower doses (Vicentini et al., 2013a).

Based on these advantages, the development of *in situ* gelling delivery systems for the local release of siRNA was proposed. The delivery system is composed of: MO (Fig. 1a and b present the structure of the two main MO used), which is responsible for the viscous liquid crystalline phase formation, propylene glycol (PG) and 0.1 M Tris buffer (pH 6.5) (tris buffer) that provides fluidity to the precursor formulation and oleylamine (OAM) (Fig. 1c), which is a cationic lipid used to confer a positive residual load to the system and then allow the formation of a complex between the system and the nucleic acids. This complex formation is important to ensure gene transfection and avoid gene degradation (Vicentini et al., 2013a).

The formation of liquid crystals is influenced by the temperature, the characteristics of the lipids and the incorporated drug or additives and the water content of the system (Chang and Bodmeier, 1998; Fong et al., 2009; Gosenca et al., 2013). Depending on the combination of these components, it is possible to obtain different types of liquid crystalline phase (e.g., lamellar, hexagonal or cubic phase). The lamellar phase consists of a linear structure of alternating lipid bilayer with water channels; this phase is less viscous and is injectable; therefore, the lamellar phase can be used for the obtainment of cubic phase due to its ability to absorb excess water from the body fluid, forming a rigid and viscous cubic phase gel. The cubic phase consists of a curved, bicontinuous lipid bilayer extending in three dimensions, separating two congruent networks of water channels; it forms with increasing water content (Larsson, 1989). With an increase in the solvent concentration, a transformation of the solvated molecules from the rod shape (lamellar phase) to a cone shape can occur. Depending on the polarity of the solvating agent and the molecule itself, the transition may result in a hexagonal or a reversed hexagonal phase (Muller-Goymann, 2004). Due to these characteristics, less viscous liquid crystalline systems can be used as *in situ* gelling delivery systems.

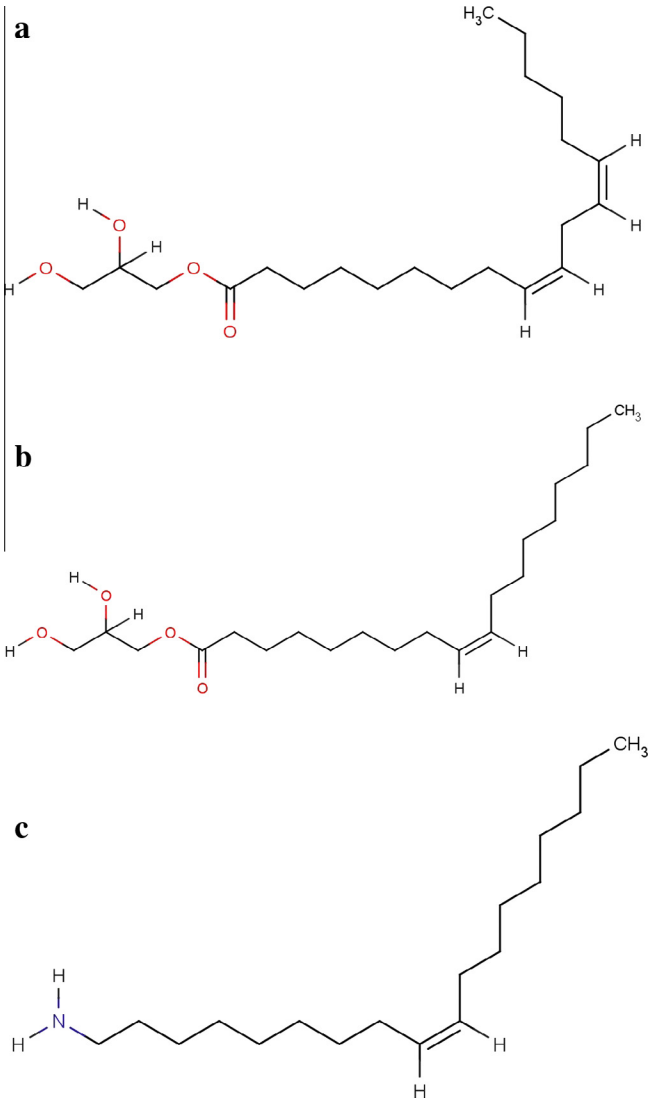

Fig. 1. Chemical structures of (a) glyceryl monolinoleate (monolinolein), (b) glyceryl monooleate (monoolein) and (c) OAM.

Because the components have important effects on the liquid crystalline phase formation, understanding the formation process is important for the development of a delivery system. Each phase has unique characteristics that will influence drug release and, consequently, the therapeutic effect of the drug. In the present study, the systems were characterized by polarized light microscopy, Small Angle X-ray Scattering (SAXS) and swelling studies in addition to studies of complexation with genes, gene stability in the systems, the ability to form a gel *in situ* and local toxicity *in vivo*. These studies were essential to define a potential delivery system for siRNA therapy.

2. Material and methods

2.1. Materials

Monoglycerides (MO, Myverol 18–92 K is composed of 93% of monoglycerides (containing 65% glyceryl monolinoleate, 23%