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Liquid Crystalline Systems Based on Glyceryl Monooleate and Penetration Enhancers for Skin Delivery of Celecoxib: Characterization, *In Vitro* Drug Release, and *In Vivo* Studies

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ABSTRACT

Celecoxib (CXB) is a widely used anti-inflammatory drug that also acts as a chemopreventive agent against several types of cancer, including skin cancer. As the long-term oral administration of CXB has been associated with severe side effects, the skin delivery of this drug represents a promising alternative for the treatment of skin inflammatory conditions and chemoprevention of skin cancer. We prepared and characterized liquid crystalline systems based on glyceryl monooleate and water containing penetration enhancers which were primarily designed to promote skin delivery of CXB. Analysis of their phase behavior revealed the formation of cubic and hexagonal phases depending on the systems' composition. The systems' structure and composition markedly affected the *in vitro* CXB release profile. Oleic acid reduced CXB release rate, but association oleic acid/propylene glycol increased the drug release rate. The developed systems significantly reduced inflammation in an aerosil-induced rat paw edema model. The systems' composition and liquid crystalline structure influenced their anti-inflammatory potency. Cubic phase systems containing oleic acid/propylene glycol association reduced edema in a sustained manner, indicating that they modulate CXB release and permeation. Our findings demonstrate that the developed liquid crystalline systems are potential carriers for the skin delivery of CXB.

Introduction

Celecoxib (CXB) is a nonsteroidal anti-inflammatory drug that acts by selectively inhibiting the enzymatic activity of cyclooxygenase-2. CXB also displays significant chemopreventive activity against several types of cancer, including ultraviolet B radiation–induced skin cancer.[1-7] As the long-term oral administration of CXB to treat inflammation has been associated with gastric and cardiovascular side effects, the skin delivery of CXB represents an interesting strategy for the treatment of skin inflammatory conditions and chemoprevention of skin cancer. This administration route provides local absorption at the inflammatory site and reduces the risk of systemic toxicity. However, the stratum corneum—the outmost skin layer—is an effective barrier to drug permeation that hinders the skin penetration of CXB, a highly lipophilic drug.

Scientists have explored several approaches to deliver CXB to the skin and increase its penetration into the skin, such as the use of penetration enhancers and the development of drug delivery systems, including microemulsions and nanoemulsions, nanostructured lipid carriers, cyclodextrins, and liquid crystalline systems.[2-11] Liquid crystalline systems are suitable vehicles for skin delivery of drugs because they are able to control drug release, improve skin penetration, incorporate hydrophilic and lipophilic drugs due to their amphiphilic nature, and protect drugs from physical degradation.[12-17]

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Table 1
Composition of the Liquid Crystalline Systems

System	Composition (%, w/w)			
	GMO/Water (70:30, w/w)	OA	PG	CXB
GMO-W	100%	0	0	0
GMO-W/CXB	98%	0	0	2%
GMO-W/OA	95%	5%	0	0
GMO-W/OA/CXB	93%	5%	0	2%
GMO-W/OA/PG(5)	90%	5%	5%	0
GMO-W/OA/PG(5)/CXB	88%	5%	5%	2%
GMO-W/OA/PG(10)	85%	5%	10%	0
GMO-W/OA/PG(10)/CXB	83%	5%	10%	2%

In this sense, in this study we propose the use of liquid crystalline systems of glyceryl monooleate (GMO) for the skin delivery of CXB. The structure of these systems consists in a complex matrix that retards diffusion and provides sustained release of drugs; for this reason, these systems have been studied as sustained drug delivery devices for several drugs and routes of administration, including skin delivery of drugs.[13,17-20] GMO is a polar lipid that forms liquid crystalline phases upon contact with water and acts as permeation enhancer by promoting ceramide extraction and enhancing lipid fluidity in the stratum corneum; hence, GMO can also increase skin permeation of drugs.[14,21,22]

Addition of solubilizers such as glycerol, propylene glycol (PG), polyethylene glycol (PEG), and ethanol, and of penetration enhancers like oleic acid (OA), can be a useful strategy to modulate drug release and skin permeation, that is, to optimize skin delivery of drugs.[22-24] As the presence of additives with different polarities can alter the packing parameter and phase behavior of liquid crystalline systems, and thereby influence the drug release pattern from these systems,[25-27] it is necessary to characterize the systems containing additives.

In the present study, we examined whether liquid crystalline systems composed of GMO and water (GMO-W), and containing the additives (penetration enhancers PG and OA), are appropriate for the skin delivery of CXB. To identify the mesophases formed, the liquid crystalline systems were characterized by polarized light microscopy and small angle X-ray scattering (SAXS). We also assessed the *in vitro* drug release profile and the *in vivo* anti-inflammatory activity of the prepared systems.

Materials and Methods

Chemicals

The glyceryl monooleate (GMO) used was a commercial preparation of monoglycerides derived from canola oil (Myverol 18-99) provided by Kerry do Brasil (Campinas, São Paulo, Brazil). CXB was purchased from Exim Pharm International (Mumbai, India), PG was obtained from Labsynth® (Diadema, SP, Brazil), and OA was acquired from Sigma-Aldrich (St. Louis, MO). All the other chemicals were of analytical grade.

Preparation of Liquid Crystalline Systems of GMO and Water

The systems were prepared by melting GMO at 40°C followed by addition of water at the same temperature, at the ratio 70:30 (w/w). Drug-loaded systems were prepared by dissolving 2.0% (w/w) CXB in the molten GMO before addition of water. OA was added to the molten GMO and PG was added to the aqueous phase, due to their solubility. The mixtures were left to stand for 24 h to reach equilibrium and formation of liquid crystalline phases. Composition of the liquid crystalline systems prepared is reported in Table 1. The GMO/water ratio was kept constant in all the systems.

Assessment of Formation of Liquid Crystalline Phases

Polarizing Light Microscopy

The liquid crystalline phases formed in all the systems were identified by macroscopic observation and polarized light microscopy analysis after 24 h, 7 days, and 14 days of preparation, according to Rosevear[28] (1954). Macroscopic aspects were evaluated by visual observation of the systems. Cubic phase presents macroscopic aspect of a transparent, rigid, and stiff gel, whereas lamellar and hexagonal phases are less viscous and opaque gels. Polarizing light microscopy was performed using an Eclipse E200 light microscope (Nikon) equipped with polarizing filters and a Moticam 2000 digital camera with the automatic image acquisition system Motic Images Plus 2.0. Complete solubilization of drug was also evidenced microscopically by the presence of drug crystals.

Small Angle X-Ray Scattering

The liquid crystalline structure of the systems was determined by SAXS measurements at 7 days after formation, using a Bruker AXS Nanostar small angle X-ray camera with a microfocus Genix 3D system (source of copper Kα radiation, $\lambda = 0.15418$ nm + focusing mirrors), 2 scatterless slits sets for collimation, and a 2D Vantec-2000 detector. The systems were placed in a sample holder between mica sheets. The acquisition time of each scattering curve was 1 h at room temperature. The intensity measurements were performed as a function of the scattering vector q ($q = [4\pi\sin\theta]/\lambda$). The sample-detector distance was 667 mm, which provided a q range of 0 to 3.0 nm^{-1}. The scattering from the mica sheets in the sample holder was removed from the scattering of each sample. The results were also corrected by a detector response, and the SAXS data were normalized to take into account the transmission for each case.

In Vitro *Drug Release Studies*

In vitro drug release studies were carried out for 24 h, at 37 ± 0.5°C, under constant stirring (50 rpm), in a dissolution test apparatus (Nova Ética Produtos e Equipamentos Científicos, Vargem Grande Paulista, SP, Brazil) adapted for semisolid samples using a hydrophobic membrane of polytetrafluoroethylene. To ensure sink conditions, isotonic phosphate buffer containing 2% (w/v) Tween 20 was used as the receptor medium. Samples of receptor medium were taken at 0.5, 1, 2, 3, 4, 5, 6, and 24 h, and the same volume was replaced. The released CXB amount was quantified at 254 nm, using the UV-VIS Femto 800 XI spectrophotometer. The release kinetics was determined by linear regression analysis of the XY scatter plot, applying the zero-order kinetic model (released drug amount vs. time), the Higuchi model (released drug amount vs. the square root of time), and the first-order kinetic model (remaining drug amount vs. time).

In Vivo *Anti-Inflammatory Effect in the Aerosil-Induced Rat Paw Edema Model*

Animals

Twenty-four male Wistar rats (6 to 8 weeks old) were housed in the Animal Facility of the Faculdades Integradas Padre Albino (FIPA; Catanduva, SP, Brazil), under controlled temperature (24°C-26°C), with a daily 12:12 h light/dark cycle, and food and water *ad libitum*. All the experimental procedures complied with the guidelines of the Institutional Animals Ethics Committee. The Animal Use and Care Committee from the Faculdades Integradas Padre Albino approved the study protocol (authorization number 15.11.27-06).