A new injectable liquid crystal-forming system for one month delivery of Leuprolide

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ABSTRACT SUMMARY
An injectable liquid crystal-forming system (LCFS) was prepared by using sorbitan monooleate (SMO) as a new liquid crystal-forming material, and its potential use in clinically available sustained-release formulation was evaluated. LCFS was prepared using SMO mixed with phosphatidyl choline and tocopherol acetate, and contained 3.75 mg of Leuprolide as a monthly dose in 90 µl of a liquid form. The semi-solid mesophase was formed from the liquid LCFS when it contacted water. The mesophase showed typical characteristics of the liquid crystalline phase, which was the hexagonal phase. in vitro pharmacokinetic study showed a sustained release of Leuprolide. When compared with a commercial depot formulation of Leuprolide, the LCFS showed a similar AUC<sub>1-24 h</sub> value and significantly reduced initial burst after subcutaneous injections in rats and dogs. The LCFS can serve as a new type of sustained-release injection formulations for its safety, ease of preparation, and sustained release properties.

INTRODUCTION
Leuprolide is a potent luteinizing hormone releasing hormone (LHRH) agonist that stimulates the release of luteinizing hormone. Leuprolide has been used successfully for the palliative treatment of prostate cancer by saturating and downregulating pituitary receptors, resulting in the suppression of testosterone production. Commercially, LHRH agonists have been formulated in controlled release PLGA microspheres administered as a depot injection lasting up to 1 month.

Recently, liquid crystal technology has emerged as a new injectable SR formulation for its sustained drug release properties. Lyotropic liquid crystal systems composed of amphiphiles can be classified into lamellar (L<sub>a</sub>), hexagonal and cubic phases based on their assembly shape. Among them, the reversed hexagonal phase (H<sub>2</sub>) and the reversed cubic phase (Q<sub>d</sub>) have been extensively investigated for their ability to control the release rate of numerous drug substances, from low-molecular-weight chemicals to macromolecular drugs (proteins, peptides and nucleic acids). Various amphiphilic liquid crystal-forming materials (LCFMs), such as glycerol monooleate (GMO), glycerol dioleate (GDO) and etc., have been reported. The drug release patterns of different types of LCFMs have been evaluated for the development of lyotropic liquid crystals as controlled release dosage forms.

The Sorbitan monooleate (SMO) (also known as Span 80) was used as a new LCFM because it has been used as a pharmaceutical excipient and regarded as safe through many medical applications. This study is the first case of applying SMO in liquid crystal technology for SR injection. SMO has advantages in terms of safety and quality control when it is applied in SR injections, because it is an injectable emulsifier that has been used in various pharmaceutical formulations.

The objectives of this study were to provide a LCFS composed of SMO for a sustained release of Leuprolide. LCFS exists as a liquid phase in the absence of aqueous fluid and forms into a liquid crystal in the presence of aqueous fluid which can be injected without producing pain or inflammations, problems with conventional formulations.

EXPERIMENTAL METHODS
The LCFS was prepared by adding and mixing a Leuprolide solution into the LCFS vehicle solution. The Leuprolide solution was prepared by dissolving 3.75 mg of Leuprolide acetate in 5 µl of DMSO. The LCFS vehicle solution was prepared by mixing SMO, phosphatidyl choline, tocopherol acetate, Tween 80 and ethanol. The final LCFS was designed to contain 3.75 mg of Leuprolide acetate for one month dose in 90 µl. The commercialized Leuplin DPS Injection® 3.75 mg (Takeda Pharmaceutical Company Limited, Osaka, Japan) was used as a reference product.

The 15 µl of the LCFS in the oil phase was added to 3 ml of triple distilled water and dispersed using a probe sonicator to form mesophase particles. The dispersed liquid crystalline phase was placed on the holey carbon-coated grid like a water film and quickly frozen at -170°C. The frozen grid was fixed in the cryo-holder and moved to the Cryo-TEM (Tecnai G2 F20 Cryo-TEM, FEI Company, Hillsboro, OR, USA) at -170°C. The sample was observed at the 80-200 kV dose.

LCFS (15 µl) was dropped and thinly spread on the glass slide. The glass slide was placed in a petri dish that contained 10 ml of triple distilled water for 15 min to form the liquid crystalline phase on the glass. The cover glass was placed slowly on the glass slide so as not to form air bubbles, and then the cover glass was sealed with silicone grease to prevent water evaporation at 25°C. All the photographs were taken under the condition of the cross-polarizer by using Polarized optical microscopy (BA 300 Pol, Motic, British Columbia, Canada).
The Leuplin DPS Injection® 3.75 mg (reference) and LCFS containing 3.75 mg of Leuprolide acetate were subcutaneously injected into the back of the rat (5 animals per group). Another in vivo test, Reference and LCFS containing 3.75 mg of Leuprolide acetate were subcutaneously injected into the back of the beagle dog (5 animals per group). Leuprolide concentrations in plasma sample taken from the SD rats and Beagle dogs were monitored for 28 days using a UPLC-MS/MS method.

RESULTS AND DISCUSSION

![Formation of liquid crystal in aqueous Fluid.](Image)

A LCFS based on SMO, phosphatidyl choline, and tocopherol acetate was developed for the subcutaneous delivery of Leuprolide. The LCFS was transformed into a gel-like mesophase by forming a lyotropic liquid crystal in 5 min after the LCFS containing SMO came in contact with PBS. As soon as the LCFS was injected into the water, it lost the flow property of a liquid and changed into a spherical semi-solid form to minimize the contact surface with water, exhibiting a semi-transparent and light yellow appearance (Figure 1).

![Determination of inner structure of LCFS.](Image)

The crystalline phase formed by SMO was observed to have mesophases with an internal curved longitudinal axis and mesophase particles with hexosome- or cubosome-like particles (Figure 2-a). The characteristic optical texture from the arrangement of the hexagonal phase can be observed using polarized optical microscopy (PLM) (Figure 2-b). All of the results from the Cryo-TEM and PLM revealed that the mesophase and mesophase particles from the LCFS with SMO exhibited typical characteristics of the liquid crystalline phase, which were classified into the hexagonal phase in terms of their structure.

As shown in Figure 3, The Reference showed a slight increase in plasma concentration around 14 days in rats and dogs due to the characteristics of PLGA formulations, but the maintenance of the steady state was not influenced. The LCFS exhibited a plasma concentration with a slowly decreasing pattern without a significant change from 7 to 28 days. Although their profiles after 7 days were slightly different due to the characteristics of each formulation, the steady state of plasma concentration in both groups was well maintained.

![Plasma concentrations of Leuprolide after subcutaneous administration of Reference and LCFS in SD rats (upper) and Beagle dogs (lower).](Image)

CONCLUSION

Consequently, the LCFS is believed to have a sufficient therapeutic effect when it is used for SR injection of Leuprolide. The LCFS composed of injectable excipients including SMO is expected to replace conventional depot injections in terms of safety, ease of preparation, and suitability of controlled release properties. It is also expected that the developed LCFS for SR injections in liquid form can be applied for the controlled release of a wide range of drugs ranging from low molecular chemical entities to high molecular drugs, such as peptides and proteins.

REFERENCES