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A novel lipid-based solid dispersion for enhancing oral bioavailability of Lycopene – *In vivo* evaluation using a pig model

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ABSTRACT

Lycopene is a potent anti-oxidant, which has been widely reported for its potential benefits at reducing the risks of certain types of cancer *e.g.* prostate cancer. The oral bioavailability of this highly lipophilic carotenoid is low and highly influenced by the extent of intestinal lymphatic uptake. The aim of this study was to develop an optimised formulation, which allows for efficient absorption following oral administration. A self-emulsifying drug delivery system (SEDDS) and solid dispersion of Lycopene were developed initially. Subsequently, a novel lipid based solid dispersion (LBSD) was designed. Processing *via* a solid dispersion approach was found to alter the solid state characteristics of Lycopene, as determined by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). The bioavailability of Lycopene was significantly increased after oral administration of LBSD to fasted pigs, relative to the commercial product (Lycovit®). A clear distinction in terms of C_{max} and AUC was observed between Lycovit® and LBSD. In conclusion, a novel LBSD formulation was developed to enhance the oral bioavailability of the model lipophilic compound, Lycopene, by enhancing dissolution in the gastrointestinal tract and promoting intestinal lymphatic uptake utilising digestible lipid excipients.

1. Introduction

Lycopene has attracted considerable attention as a natural chemo-preventative agent through various properties including its potent antioxidant properties, inhibition of cancer cell proliferation, and decreased lipid oxidation (Boileau et al., 2003; Giovannucci, 2005; Hadley et al., 2003; Nahum et al., 2001). Numerous epidemiological studies have demonstrated that high dietary intake of Lycopene, (primarily tomato-based food products) is associated with reduced risk of prostate cancer (Etminan et al., 2004; Giovannucci et al., 1995; Kim et al., 2003). However, there have also been a number of studies that have failed to clearly demonstrate a chemo-preventative effect of Lycopene (Hayes et al., 1999; Kolonel et al., 2000). Critically, a key limitation of studies evaluating potential relationships between dietary Lycopene intake and prostate cancer risk is that Lycopene levels in plasma or tissues are not strongly correlated with overall dietary consumption of Lycopene (Giovannucci, 2005; Michaud et al., 1998). Lycopene absorption from dietary foodstuffs is estimated to be low, variable and highly influenced by the composition of the meal (Wei and Giovannucci, 2012). For example, systemic uptake of Lycopene was 2.5 fold higher from tomato paste compared to fresh tomatoes (Gartner et al., 1997). Few studies have explored the design of oral formulations containing Lycopene, and there is a need for an optimised formulation, which allows for efficient and reproducible bioavailability, which would then serve as the basis for future investigations into the potential benefits of this potent antioxidant in humans.

Lycopene is a highly lipophilic carotenoid ($C\log P = 17.6$), with poor aqueous solubility, and previous studies have predicted that Lycopene will display solubility rate limited absorption characteristics (Faisal et al., 2010; Vertzoni et al., 2006). Hence formulation approaches which enhance solubility of Lycopene within the gastrointestinal tract (GIT) are considered crucial to increasing oral absorption. A number of formulation approaches have been utilised to enhance solubility of poorly soluble drugs in the GIT, such as particle size reduction, or modifications of crystal habit to enhance dissolution (Leuner and Dressman, 2000). Solid dispersion formulations (SD), where the drug is dispersed within an inert carrier matrix, have also attracted considerable interest (Craig, 2002). The main interest in SD approaches have centred on attempts to either reduce particle size to a near molecular level and/or produce an amorphous form of the drug dispersed within the carrier, with the aim of increasing the dissolution rates relative to the pure crystalline form of the drug (Chiou and Riegelman, 1971; Corrigan, 1985). In addition, advances in the processing techniques and the range of carrier types have broadened the potential uses and applications of SD technology, as previously

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reviewed (Vasconcelos et al., 2007). Gelucires ® are one such solid carrier material, consisting of saturated polyglycolized glycerides, which have attracted much attention as a SD carrier to enhance oral bioavailability of poorly soluble drugs (Aungst et al., 1997; Barker et al., 2003). Gelucire excipients are solid waxy materials of saturated polyglycolized glycerides consisting of mono-, di-, and tri-glycerides and of mono- and di-fatty acid esters of polyethylene glycol. For example, Gelucire 44/14 displays surface active properties, which may further enhance dissolution by enhancing wetting and reducing surface tension between the drug and dissolution medium (Chambin and Jannin, 2005; Jannin et al., 2008).

Alternatively, lipid-based formulation approaches may be successfully employed to enhance drug solubilisation within the gastrointestinal tract (O'Driscoll and Griffin, 2008). Self-emulsifying drug delivery system (SEDDS) consist of blend of lipids and surfactants which self-emulsify on dispersion in the gastrointestinal media to form fine o/w microemulsions (Gershanik and Benita, 2000). Highly lipophilic drugs, which are dissolved in the SEDDS pre-concentrate, are therefore solubilised within the o/w microemulsion formed in the GIT, and ideally maintained in a solubilised state on transfer into the pre-absorptive bile salt mixed micellar state. Essentially, for poorly water soluble drugs, which are dissolution-rate limited, a SEDDS approach has the advantage of avoiding drug dissolution as the drug is effectively solubilised throughout intestinal transit (Pouton and Porter, 2008). The choice of lipid excipients in the SEDDS blend is therefore critical to maintaining solubilisation of the drug on dispersion in intestinal fluid, and avoid drug precipitation during GIT transit (Fei et al., 2013; Stillhart et al., 2013).

The aim of this study was to develop a formulation strategy to enhance the oral bioavailability of the highly lipophilic model compound, Lycopene. Previous studies have shown that the intestinal lymphatic route is the major uptake mechanism of Lycopene from the gastrointestinal tract, and therefore a lymphotropic formulation strategy may enhance overall bioavailability (Faisal et al., 2010). Long chain triglycerides (LCT) have been previously shown to enhance intestinal lymphatic transport of highly lipophilic drugs (Caliph et al., 2000; Khoo et al., 2003). Therefore, initial studies were centred on the design of a LCT-SEDDS formulation. A Gelucire-based SD was also designed with the objective of enhancing dissolution characteristics of Lycopene. Characterisation of the drug in the various formulations was performed by differential scanning calorimetry (DSC), X-ray diffraction (XRD), and drug release was quantified by dissolution studies. A novel lipid based solid dispersion (LBSD) formulation was subsequently developed. The bioavailability of Lycopene in the LBSD was evaluated, relative to commercial Lycopene formulation (Lycovit®, BASF), after oral dosing to fasted pigs.

2. Materials and methods

2.1. Materials

Lycopene was purchased from Xian Terra Biochem Co., Ltd, China (*all trans* Lycopene >91%). Lycovit® (10% Lycopene) and Cremophor RH 40 were kindly donated by BASF Health& Nutrition. Gelucire 44/14 was received from Gattefossé (St. Priest, France). Ethyl-b-apo-carotene-8-oate, the internal standard (IS), was purchased from CaroteNature (Lupsingen, Switzerland). Lecithin (Lipoid EPC, >98% pure) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). All chemicals and solvents were of HPLC grade and purchased from Sigma-Aldrich (St Louis, USA).

Table 1
Composition and drug loading of different formulations.

Formulation	Composition	Drug load
Lycopene	Crystalline drug substance.	50 mg
SEDDS	40% LCT 40%Tween 85 20%Cremophor RH	0.26 mg/g
Solid dispersion	100% Gelucire	50 mg/g
LBSD	20% LCT 20% Tween 85 10% Cremophor RH 50% Gelucire	50 mg/g
Lycovit®	10% Lycopene, encapsulated in gelatine beadlets.[a]	100 mg/g

[a] Also contains sucrose, corn starch, alpha tocopherol (http://www.basf.cl).

2.2. Preparation of the drug loaded SEDDS

Screening of the self-emulsifying properties was conducted by establishing a pseudo ternary phase diagram with systems composed of long chain triglyceride (LCT) (Olive oil), a surfactant (Cremophor RH), a co-surfactant (Tween 85), after dispersion (1:100 v/v) in water (Griffin and O'Driscoll, 2006). A SEDDS formulation, comprising 40% LCT, 20% Cremophor RH, and 40%w/v Tween 85, was chosen, on the basis of its high composition of LCT and the ability to form a stable isotropically clear microemulsion on dilution with water (Table 1). The SEDDS was prepared by weighing exact quantities of each excipient into a screw cap glass tube followed by vortexing to allow complete mixing. After overnight incubation at 37 °C, isotropic mixtures were assessed for the efficiency of self-emulsification. Droplet size and polydispersity index (PI) of the microemulsion that formed on dilution were determined by using Malvern Zetasizer instrument (Nano-ZS, UK). The solubility of Lycopene in the SEDDS pre-concentrate was determined as previously described (Faisal et al., 2010).

2.3. Preparation of the drug loaded solid dispersions

A Solid dispersion (SD) of Lycopene in Gelucire 44/14 was prepared using a conventional solvent evaporation method, at the weight ratio of 1:20. Gelucire was dispensed into a glass vial and placed in a water bath held at approximately 55 °C. Lycopene and molten carrier were then dissolved in a minimum volume of dichloromethane (10 mg Lycopene/mL) and heated briefly to 40 °C. The solution was transferred to rotary evaporator and the solvent was evaporated under vacuum at 40 °C. The preparation was cooled and finally stored at −80 °C.

2.4. Preparation of the drug loaded – lipid based solid dispersions (LBSD)

A novel formula containing a combination of Gelucire 44/14 and SEDDS was prepared (Table 1). Solid dispersion (10 g) of Lycopene and Gelucire 44/14 at the weight ratio of 1:10 was prepared by a solvent evaporation method, as described in Section 2.3. Subsequently after solvent removal, 10 g of SEDDS, containing 2.6 mg Lycopene as described in Section 2.2, was added quickly to the dispersion and mixed well with spatula on water bath and the mixture was then rapidly cooled and finally stored at −80 °C.

2.5. Characterisation of formulations

2.5.1. Differential scanning calorimetry

Thermal analysis was performed on a DSC Q1000 (TA Instruments, UK). Samples were accurately weighed into aluminium pans