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Research Paper

Bioavailability of lycopene in the rat: the role of intestinal lymphatic transport

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Abstract

Objectives As a natural antioxidant derived from dietary sources, lycopene has attracted considerable attention as a potent chemopreventative agent. Lycopene is an extremely lipophilic compound and absorption from dietary sources is estimated to be low and highly variable. As a result, plasma lycopene concentrations are poorly correlated with dietary intake of lycopene rich food stuffs. The development of an oral formulation remains a challenge that requires a better understanding of the mechanisms involved in the intestinal absorption of this compound.

Methods The solubility of lycopene in simulated physiological fluids and bile salt mixed micelle formulations was determined. The extent of intestinal lymphatic transport and the absolute bioavailability of lycopene from a range of biorelevant media was evaluated in a mesenteric lymph duct cannulated anaesthetised rat model.

Results The absolute bioavailability of lycopene after 8 h was $1.85 \pm 0.39\%$. The overall extent of the intestinal lymphatic transport was in the range of 0.6–3.4% of the administered dose. A strong positive correlation ($r^2 > 0.9$) between intestinal lycopene levels and intestinal triglyceride levels was demonstrated.

Conclusions The intestinal lymphatic route is the major uptake mechanism of lycopene from the gastrointestinal tract. Lycopene transport in intestinal lymph was closely associated with triglyceride transport in the lymph. Formulation strategies designed to promote intestinal lymphatic uptake, such as lipid-based formulations containing long-chain fatty acids (LCFA) or lecithin, may serve to enhance oral bioavailability of lycopene.

Keywords gastrointestinal absorption; intestinal lymphatics; lipid based formulations; lycopene bioavailability

Introduction

There are numerous scientific studies supporting the role of dietary lycopene in reducing the risk of certain malignancies, particularly prostate cancer.[1–3] In 2005, Giovannucci suggested a significant 25–30% reduction in prostate cancer risk based on a review of epidemiological studies wherein high plasma lycopene levels and risk of prostate cancer were compared.[4] In addition, lycopene has been found to be the most potent antioxidant among the carotenoids *in vitro* and has also demonstrated anti-proliferative effects in induced cancer animal models.[2,5] Widespread agreement on the proposed chemopreventative effects of this 'nutraceutical' has suffered, however, as a number of dietary-based epidemiological studies, which have compared patients with a high intake of lycopene rich foods (e.g. tomatoes) and prostate cancer risk, have yielded less supportive results, with some but not all demonstrating a protective effect.[3] A key limitation to dietary-based studies, however, is the variability in lycopene absorption from various sources of food stuffs, such as tomatoes and tomato-based products.[6,7] In particular, and unlike other carotenoids, lycopene levels in plasma or tissues do not appear to be well correlated with overall dietary consumption of lycopene.[8] Furthermore, it has also been suggested that in some of these key dietary studies that bioavailability of lycopene from certain foodstuffs may be too low to demonstrate an association.[7]

Lycopene is the most abundant carotenoid present in human plasma, accounting for approximately 50% of all plasma carotenoid content.[9] However, the extent of absorption from dietary sources is estimated to be low and highly variable.[10,11] It is a C40 acyclic hydrocarbon, which displays extreme lipophilic properties (clog $P > 17$). The mechanisms by which lycopene is absorbed from the gastrointestinal tract are poorly explored. Precise

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data on the bioavailability of lycopene is limited to date as the majority of studies have been conducted using crude food-based dietary supplements. Studies evaluating the factors influencing bioavailability of pure compound are only recently being reported.[12,13] A mechanistic evaluation of the solubility in biorelevant media, uptake from the gastrointestinal tract and absolute bioavailability of this compound remain to be explored and are essential to guiding the design of an optimal oral formulation strategy that will enhance oral bioavailability.

Absorption of drugs from the gastrointestinal tract generally involves uptake into enterocytes followed by transport to the systemic circulation via either the hepatic portal blood or intestinal lymphatic routes. The intestinal lymphatic pathway plays a pivotal role in the gastrointestinal absorption of dietary lipids and highly lipophilic xenobiotics. Numerous drugs have been described that reach the systemic circulation, at least partly, via the lymphatic route, in general by association with triglyceride-rich lipoproteins synthesised in the intestinal enterocytes. For these compounds, lipid-based formulations have been shown to promote intestinal lymphatic uptake.[14] The current work is aimed at evaluating the factors influencing the absorption of lycopene. The solubility of lycopene in a range of biorelevant and physiological buffer media was evaluated initially. Subsequently, the absorption mechanisms of lycopene were investigated in the rat model, which has proven a reliable predictor of the absorption characteristics of drugs in humans.[15,16] The absolute bioavailability of lycopene following intraduodenal dosing in an anaesthetised rat model was assessed with reference to an intravenous control. The mesenteric lymph duct of the rats was cannulated to facilitate quantification of the extent of intestinal lymphatic transport of lycopene. The study design, involving dosing in both sham-operated and mesenteric lymph-cannulated rats, facilitates an indirect estimation of the impact of intestinal lymphatic transport on absolute bioavailability of lycopene. Finally, the impact of formulation of lycopene in a series of lipid-based micellar systems was examined in an attempt to further elucidate the absorption mechanisms involved.

Materials and Methods

Materials

Lycopene was extracted from the commercial product Lycovit (10% lycopene), which was kindly donated by BASF. Lycovit (0.5 g) was ground in a glass mortar, under dark conditions, followed by addition of hexane and further grinding. The clear supernatant was collected and evaporated under vacuum at 35°C. The residue was collected and stored at −80°C. The purity was confirmed by UV spectroscopy versus a standard all-*trans*-lycopene (purchased from CaroteNature, Lupsingen, Switzerland). The $E^{1\%}$ value, which is the absorbance of 1 g all-*trans* lycopene solubilised in 100 ml hexane and measured at wavelength 472 nm, was determined and found to be similar to reported values.[17] In addition, C18 HPLC UV chromatography confirmed a purity in the sample of >95% compared with the standard lycopene sample. The isomer profile of extracted lycopene was evaluated on a C30 HPLC system, which facilitates a separation and quantification of lycopene isomers. The lycopene extract was found to be primarily composed of all-*trans* (70%) and 5-*cis* isomers (23%), which is similar to that reported for Lycovit.[18] All chemicals and solvents were of HPLC grade and purchased from Sigma-Aldrich (St Louis, USA). Ethyl-β-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).

Preparation of biorelevant media

Simple micellar systems were prepared by adding 2% w/v sodium cholate (NaC) in approximately one-tenth of the final volume of isotonic phosphate buffer (pH 6.8) at 37°C with constant stirring. When the bile salt was completely dissolved, the solution was made up to final volume then stirred for a further 30 min. For mixed micellar systems, the quantity of fatty acid was added drop-wise (at 37°C under continuous stirring) over 30 min to a simple micellar solution. Mixed micellar systems, containing either oleic acid (a C18 long-chain fatty acid (LCFA)) or decanoic acid (a C10 medium-chain fatty acid (MCFA)) were prepared. When all the fatty acid had been solubilised, the solution was made up to the desired volume and stirred for a further 30 min. All solutions were checked for transparency in front of a strong light and allowed to equilibrate at room temperature for at least 12 h.

Fasted-state simulated intestinal fluid (FaSSIF) containing 3 mM sodium taurocholate (NaTC) and 0.75 mM lecithin in a pH 6.5 phosphate buffer and fed-state simulated intestinal fluid (FeSSIF) containing 15 mM NaTC and 3.75 mM lecithin in a pH 5.0 acetate buffer were prepared as previously reported.[19,20]

Determination of solubility of lycopene in biorelevant media

Excess lycopene (2 mg) was added to 2 ml of biorelevant medium (i.e. FaSSIF/FeSSIF, simple and mixed bile salt micelles) in an amber glass tube. The mixture was vortexed for 5 min. Preliminary equilibrium solubility studies involving periodic sampling over 48 h indicated that equilibrium solubility was achieved rapidly (i.e. within 15–30 min). However, the concentration of lycopene in media appeared to decrease at times in excess of 4–6 h, reflecting possible isomerisation and or degradation of lycopene in solution. As a result, an estimation of relative solubility was obtained by equilibrating the sample for 60 min in a thermostatted water bath (25°C) while shaking. Samples were withdrawn and rapidly filtered through a millipore filter (0.2 μm). The supernatant was quantified by UV spectroscopy at 472 nm. All experiments were performed under dark conditions and at ambient temperature. At least three determinations were made per sample.

Surgical procedures

All animal experiments were performed in accordance with EU directive 86/609 (as implemented in Ireland by Statutory Instrument 17/9) in association with Biological Service Unit

in University College Cork, which is registered with the Department of Health and Children. Male Wistar rats (Harlan, Huntingdon, UK), 275–300 g, were fasted for 24 h before surgery with free access to water. The rats were anaesthetised for the duration of the experiment by intraperitoneal injection of 0.3 ml of 60 mg/kg pentobarbital. The duodenum and mesenteric lymph duct were cannulated as previously described.[21] A polyethylene tube was implanted intraduodenally 1 cm below the pylorus. Rehydration via an intraduodenal infusion of normal saline at a flow rate of 2 ml/h was initiated for 0.5 h post-surgery. Lycopene solubilised in the test solution was administered by intraduodenal infusion at a rate of 2 ml/h over 2 h (i.e. total dose of 4 ml). Normal saline was then infused at a rate of 2 ml/h for the remaining 6 h of the experiment to rehydrate the rat. Lymph was collected hourly for 8 h (i.e. 2 h dosing and 6 h post-dosing periods), in pre-weighed cooled glass tubes containing anticoagulant (heparin). Non-lymph-cannulated rats were sham operated in terms of the mesenteric lymph duct cannulation and had an intraduodenal cannula (for rehydration) inserted, as described above. Serial blood samples (0.25 ml) were taken from both sham and cannulated groups by cardiac puncture at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 h after commencement of drug administration. At the end of the experiment, the rat was euthanised by intra-cardiac injection of 0.5 ml of pentobarbital sodium (60 mg/kg).

For intravenous administration of lycopene, the femoral vein and artery were exposed through an incision in the right groin. The femoral vein was cannulated with polypropylene tubing and connected to an infusion pump for a constant infusion of isotonic saline at rate of 2 ml/h. The femoral vein was also used for lycopene dosing. A bolus intravenous dose of lycopene (10.14 ± 1.5 μg) was administered by dissolving lycopene in 1.5 ml of 2%w/v Cremophor EL solution. A second cannula was implanted into the right femoral artery for blood samples collection. Blood samples were taken at 5, 10, 15, 30, 45, 60, 90 and 120 min and at 1-h intervals thereafter during the rest of experiment time. Blood samples were centrifuged at 5750 rev/min for 4 min at 4°C.

Analysis

The plasma or lymph sample (200 μl) was transferred to an Eppendorf tube and 50 μl of 67 ng/ml of IS (solubilised in ethyl acetate) was added and vortexed for 20 s. For protein precipitation, 200 μl of absolute ethanol, containing 0.1% w/v butylated hydroxyl toluene (BHT), was added to the previous solution and the mixture was vortexed for 20 s. A 200-μl volume of ultra- pure water was added to the deproteinised mixture and vortexed again. Ethyl acetate (1 ml) containing 0.1% w/v BHT was added and the new mixture was vortexed for 1 min. The tube was centrifuged at 5750 rev/min for 5 min at 4°C. The clear organic phase was collected and another 1 ml of ethyl acetate was added to the residue in the tube, vortexed for 1 min and centrifuged again. The clear organic solution was collected and added to the first one and evaporated to dryness under nitrogen stream at room temperature. The residue was reconstituted in 50 μl of ethyl acetate, vortexed for 20 s and made up to 0.5 ml with acetonitrile. This solution was injected onto an HPLC system equipped with a C18 column equilibrated with a mobile phase composed of acetonitrile–methanol (50 : 50 v/v) as previously reported.[22] The detection was performed at 472 nm.

The validity of the HPLC assay for both lycopene and IS were established by examination of the linearity of response, reproducibility of standard curve and extraction recovery. The analysis of lycopene in plasma and lymph exhibited excellent linearity ($r^2 \geq 0.999$) over the concentration range of 1–500 ng/ml. The limit of quantitation (LOQ) was determined to be 4.5 ng/ml. The extraction recoveries of lycopene and internal standard, from both lymph and plasma, were all in excess of 86%.

Lymph triglyceride (expressed as mg equivalents of C18 long-chain triglyceride (LCT)) was determined using a Triglyceride Enzymatique PAP 150 assay (bioMeirieux) as described previously.[21]

Data analysis

The AUC for lycopene after intraduodenal administration was obtained using the linear trapezoidal rule from time zero to the last measured time point. The bioavailability in non-lymph-cannulated rats after 8 h was estimated from the ratio of the dose-normalised AUCs after intraduodenal and intravenous administration as follows:

$$F = \frac{AUC_{i.d.}^{0\rightarrow 8hr}}{AUC_{i.v.}^{0\rightarrow 8hr}} \times \frac{Dose_{i.v.}}{Dose_{i.d.}} \quad (1)$$

The extent of lymphatic transport was calculated using the concentration of drug found in each lymph sample, multiplied by the volume of the lymph produced per hour, and expressed as a cumulative percentage of the dose. The value of k_{el} was estimated from the slope of the log transformed plasma concentration–time data. The volume of distribution (Vd), clearance (Cl) and apparent biological half life were calculated as follows:

$$\mathrm{Cl} = \mathrm{Dose}/\mathrm{AUC}_{0\rightarrow 8} \quad (2)$$

$$\mathrm{Vd} = \mathrm{Dose}/\mathrm{AUC}_{0\rightarrow 8} \times k_{el} \quad (3)$$

$$t_{\frac{1}{2}} = 0.693/k_{el} \quad (4)$$

Student's *t*-test was used to determine the statistical significance ($P < 0.05$) of calculated results between the experimental groups.

Results

Solubility of lycopene in biorelevant media

The solubility of lycopene in a range of biorelevant media was examined (Table 1). FaSSIF blank is a simulated intestinal buffer (pH 6.5) that contains no bile/lipid components. As expected, lycopene did not display any quantifiable solubility in FaSSIF blank (LOQ = 4.5 ng/ml). FaSSIF and FeSSIF are considered to be physiologically relevant buffer media, containing both sodium taurocholate (NaT) and phospholipids (lecithin) at physiologically relevant fasted- and fed-state concentrations, respectively. The

Table 1 Solubility of lycopene in a range of biorelevant media

Lipid-based formulation	Solubility (mg/l)
Fasted-state simulated intestinal fluid blank (FaSSIF blank)	<LOD
Fasted-state simulated intestinal fluid (FaSSIF)	7.29 ± 1.88
Fed-state simulated intestinal fluid (FeSSIF)	12.79 ± 2.07
Bile salt simple micelles – 40 mM sodium cholate (NaC)	1.38 ± 0.12
MCFA bile salt mixed micelles – 40 mM NaC : 40 mM decanoic acid	9.185 ± 1.57
LCFA bile salt mixed micelles – 40 mM NaC : 40 mM oleic acid	20.28 ± 0.56

Solubility of lycopene was determined after mixing for 60 min at 22°C (mean ± SE, $n = 3$).

solubility of lycopene was found to be significantly higher in FeSSIF than in FaSSIF ($P < 0.05$).

Pharmacokinetics of lycopene in the rat

Figure 1 represents the lycopene plasma profile in anaesthetised rats following intravenous administration of 10.14 ± 1.5 μg lycopene. The dose chosen reflected a 1.5-ml bolus dose of a solubilised lycopene in 2% Cremophor EL solution. The intravenous plasma concentration profile of lycopene decreased exponentially with time, and the data appears to closely fit a mono-exponential process ($r^2 > 0.99$). The $AUC_{0\rightarrow8}$ of lycopene was calculated to be 1.715 ± 0.445 mg · h/l. The apparent biological t½ was estimated to be 1.17 ± 0.06 h; the volume of distribution (V_d) was 34.24 ± 7.6 ml/kg body weight and the clearance (Cl) was calculated as 19.89 ± 3.46 ml/h · kg body weight.

The plasma concentration–time profiles following intraduodenal dosing in lymph-cannulated and non-lymph-cannulated rats are presented in Figure 2. Four millilitres

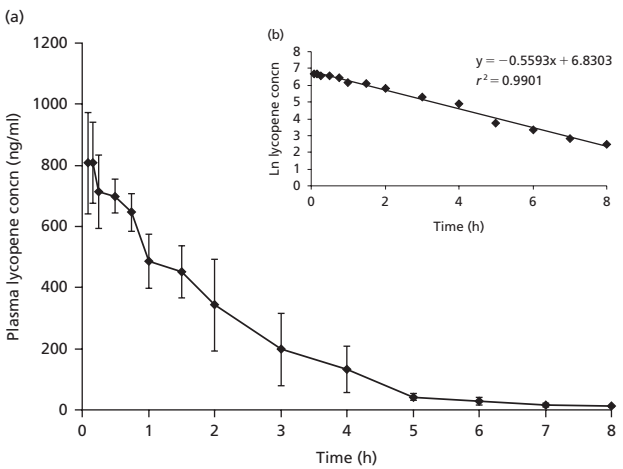

Figure 1 Lycopene plasma concentration (ng/ml) versus time profiles in anaesthetised rats. Data are presented in normal (a) and semilog scales (b). Lycopene (10.14 ± 1.5 μg/ml) solubilised in 1.5 ml of a 2%w/v Cremophor EL solution was administered by bolus intravenous dosing. Data are means ± SE, $n = 4$

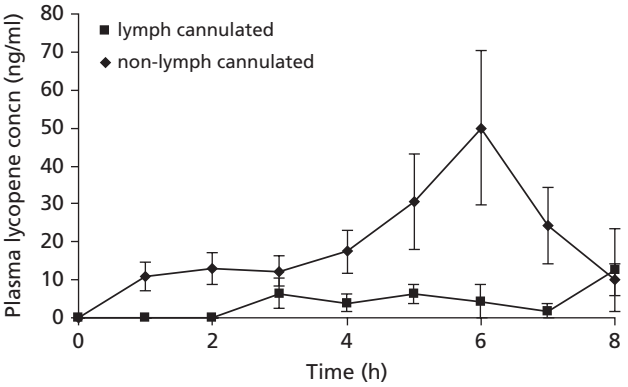

Figure 2 Lycopene plasma concentration (ng/ml) versus time profiles in lymph-cannulated and non-lymph cannulated rats after intraduodenal administration of lycopene solubilised in FeSSIF. Lycopene was administered in a dose of 53.4 ± 4.4 μg/ml. Data are means ± SE, $n \geq 4$

of a saturated solution of lycopene solubilised in FeSSIF, equivalent to a dose of 53.4 ± 4.4 μg of lycopene, was administered intraduodenally over 2 h followed by 6 h rehydration with saline. This experimental format was designed to simulate a normal intestinal digestion/transit wherein a gradual presentation of media from the stomach is released into the upper small intestine (i.e. over 2 h). The plasma $AUC_{0\rightarrow8}$ of lycopene in sham-operated rats (i.e. non lymph cannulated) was 0.187 ± 0.039 mg · h/l and the absolute bioavailability of lycopene after 8 h was estimated to be 1.84 ± 0.39% of the administered dose.

The plasma $AUC_{0\rightarrow8}$ of lycopene calculated in lymph-cannulated rats was 0.029 ± 0.007 mg · h/l, which was significantly lower than the corresponding AUC in non-lymph-cannulated rats ($P < 0.05$). The bioavailability calculated in non-lymph-cannulated rat (F_{total}) reflects the percentage of the administered dose reaching the systemic circulation by both portal ($F_{portal} = 0.294 \pm 0.073\%$) and lymphatic transport (F_{lymph}) route (i.e. $F_{total} = F_{portal} + F_{lymph}$). In lymph-cannulated rats, the bioavailability determined reflects absorption of lycopene via the portal route alone (F_{portal}), as the intestinal lymph is collected and drained. Therefore, the overall percentage of the administered dose that is transported lymphatically can be estimated from $F_{lymph} = F_{total} - F_{portal} = 1.55 \pm 0.4\%$. These results indicate that cannulation and removal of the intestinal lymphatic transport route resulted in an 84% decrease in the overall systemic absorption of lycopene. This would indicate that the intestinal lymphatic transport route is the major uptake mechanism of lycopene from the gastrointestinal tract.

Intestinal lymphatic transport of lycopene

The lymphatic transport of lycopene, expressed as the cumulative percent of the administered dose, after intraduodenal administration in a range of biorelevant media, is presented in Figure 3 with a summary of the lymphatic transport data of lycopene presented in Table 2. The greatest extent of intestinal lymphatic transport was observed for the FeSSIF media, where 3.4 ± 1.14% of the administered dose was recovered in the intestinal lymph. In lymph-cannulated

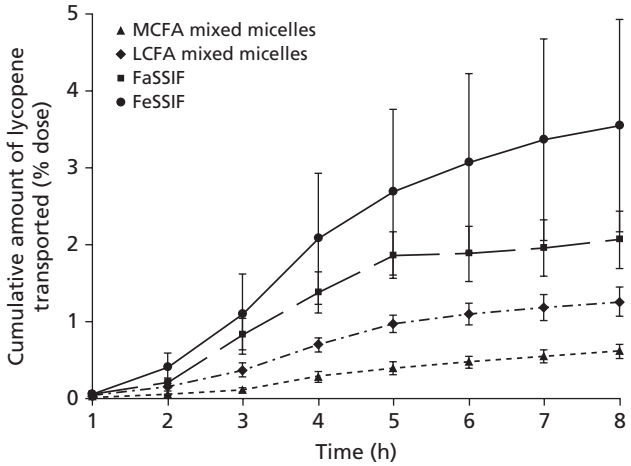

Figure 3 Cumulative percent of the dose of lycopene transported in rat intestinal lymph as a function of time. Lycopene was administered intraduodenally solubilised in a range of biorelevant media: MCFA mixed micelles; LCFA mixed micelles; FaSSIF; and FeSSIF. Data are means ± SE, $n \geq 5$

rats, it was estimated that 3.69% of the administered dose of lycopene was absorbed systemically (i.e. $F_{total} = F_{lymph}$ (3.4%) + F_{portal} (0.29%)), with the fraction of dose transported in the lymphatics being the major determinant of overall bioavailability.

LCFA mixed micelles promoted the intestinal lymphatic uptake of lycopene to a significantly greater extent (1.37 ± 0.24%) than MCFA mixed micelles 0.61 ± 0.09% ($P < 0.005$). LCFA bile salt mixed micelles (containing 40 mg of LCFA) resulted in an increase in intestinal triglyceride of 38.2 mg (Table 2). By comparison, MCFA bile salt mixed micelles (containing 40 mg of MCFA) resulted in an increase of 16.5 mg in intestinal triglyceride. Hence, MCFA are less effective at stimulating intestinal triglyceride-rich lipoprotein (TRL) synthesis.

The cumulative extent of intestinal lymphatic transport of lycopene in FaSSIF and FeSSIF over 8 h was 2.52 ± 0.25% and 3.4 ± 1.14%, respectively. While the differences did not reach statistical significance ($P = 0.16$), lymphatic transport of lycopene was consistently higher in the fed state than in the fasted-state intestinal media. Interestingly, the FaSSIF and FeSSIF formulations resulted in greater extents of intestinal lymphatic transport of lycopene compared with the bile salt mixed micelle vehicles (Table 2).

Discussion

Solubility of lycopene in biorelevant media

The results of the solubility study confirm the impact of the higher lipid composition of fed-state media on increasing lycopene solubility, and broadly support reports of increased bioavailability of lycopene following ingestion of a high fat content meal.[23,24] The values obtained for lycopene solubility were higher than those reported by Vertzoni *et al.*[13] where a 99% *trans* grade of lycopene was used. The higher solubility obtained reflects the higher *cis* content of lycopene used in the current study (i.e. 23% *cis* isomer). *Cis* isomers of lycopene display a higher solubility than all-*trans* isomers.[25]

For highly lipophilic compounds, such as lycopene, the degree of solubilisation in biorelevant media is a useful indicator of the capacity of gastrointestinal fluids to solubilise the compound, and hence a predictor of the maximum absorbable dose available. If it is assumed that in humans the maximum total volume of gastrointestinal fluids available during gastrointestinal transit is ~1.5 l, then it can be calculated, using the solubilities estimated for lycopene in FaSSIF and FeSSIF (Table 1), that the dose of pure lycopene that will be solubilised during gastrointestinal transit will be 10-18 mg.[26] While a common dose range of 10–30 mg/day is reported for lycopene, these findings suggest that doses of pure lycopene in excess of 18 mg are likely to display inadequate solubilisation within the volume of gastrointestinal fluids available and hence display solubility rate limited absorption characteristics. This finding of solubility rate limited absorption may be used to partly explain the observations of non-linear absorption characteristics reported for lycopene in humans at doses 10–120 mg.[8,27]

In the small intestine, co-administration of food or lipids leads to the secretion of bile and digestive enzymes that produce a complex mixture of intestinal colloidal phases, designed to facilitate the digestion and absorption of dietary lipids.[28] The solubilising capacity of bile salt mixed micelles is significantly higher than that of simple micelles. This superior solubilisation by mixed micelles is of relevance physiologically as the presence of the end products of lipid

Table 2 Summary of lymphatic transport kinetics after intraduodenal administration of lycopene solubilised in a range of biorelevant media to lymph-cannulated rat

	FaSSIF	FeSSIF	LCFA bile salt mixed micelle	MCFA bile salt mixed micelle
Dose (μg)	31.02 ± 3.03	45.14 ± 2.53	78.87 ± 5.47	33.47 ± 4.29
C_{max} (ng/ml)	322.51 ± 61.69	642.91 ± 203	330.89 ± 43.16	66.48 ± 27.15
T_{max} (h)	3.86 ± 0.40	3.83 ± 0.31	3.5 ± 0.22	3.67 ± 0.21
% Dose transported in lymph at 8 h	2.52 ± 0.25%	3.4 ± 1.14	1.37 ± 0.24	0.61 ± 0.09
Cumulative mass of total triglyceride in lymph at 8 h (mg)[a]	21.31 ± 1.41	27.82 ± 4.41	52.62 ± 4.66	30.93 ± 2.24
Cumulative exogenous triglyceride transport in lymph at 8 h (mg)[b]	6.91 ± 3.26	13.42 ± 4.91	38.21 ± 5.14	16.52 ± 3.11

[a]Representing endogenous and exogenous lipid. [b]Attributable to exogenous lipid (i.e. total–endogenous) where endogenous lipid was estimated to be 14.4 ± 2.16 mg by perfusing saline in a control group of rats. Data are means ± SE, $n \geq 5$.

digestion (e.g. free fatty acids) in the intestine will not only stimulate the bile acid secretion, but also increase the solvent capacity by formation of mixed micelles. Pouton and co-workers have also reported that the enhancement in solubility by the presence of lipolytic products in bile salt micelles is far greater than the solubility enhancement observed for simple micelles.[29] Mixed micelles have a larger lipophilic core, allowing more lycopene to be taken up into the core, ultimately leading to higher solubilisation of lycopene. The enhanced solubility of the mixed micelles is highly dependent on the type of fatty acid. Relative to simple bile salt micelles, LCFA serves to increase the solubilising capacity approximately ~20 fold compared with an approximate 9-fold increase for the MCFA bile salt mixed micelle systems (Table 1). Kossena *et al*. have similarly reported on a superior solubilisation capacity of mixed micelles formed from long-chain lipids for highly lipophilic drugs versus mixed micelles formed with medium-chain lipids.[30,31] Hence, in terms of optimising oral bioavailability of lycopene, formulations comprising long-chain triglyceride would appear to be favourable in terms of maximising solubilisation of lycopene within the gastrointestinal tract.

Pharmacokinetics of lycopene in the rat

To the best of our knowledge, this is the first published report on the pharmacokinetics of lycopene following intravenous administration in the rat. Vertzoni *et al*.[13,22] performed an intravenous study in dogs, although the resulting lycopene plasma concentrations were highly variable and could not be fitted to pharmacokinetic models. From our data it would appear that lycopene is relatively rapidly cleared from the plasma compartment over 8 h following a single bolus dose in rats. Given the reports of a prolonged elimination half-life for lycopene in dogs (~36 h) and humans (28–62 h), it is likely that the rapid clearance from rat plasma in the 8-h sampling time reflects a combination of re-distribution to peripheral tissues and elimination.[32,33] It is reported that following distribution into certain peripheral tissues, turnover is particularly slow.[27] However, as plasma levels have dropped below quantifiable levels within 8 h, it was not possible to determine such a prolonged elimination phase in this rat model.

A number of studies have predicted the bioavailability of lycopene to be low.[34,35] However, studies determining the absolute oral bioavailability of lycopene, by reference to an intravenous profile, are lacking. Tang *et al*.[35] used a deuterated lycopene source to evaluate the quantity of newly absorbed lycopene into plasma and, while the methods used are quite different, a bioavailability estimate of 1.2% in humans is similar to the absolute bioavailability determined in the current study.

Intestinal lymphatic transport of lycopene

The lymphatic transport of lycopene, expressed as the cumulative percent of the administered dose, after intraduodenal administration in a range of biorelevant media was studied. The greatest extent of intestinal lymphatic transport was observed for the FeSSIF media, where $3.4 \pm 1.14\%$ of the administered dose was recovered in the intestinal lymph. In lymph-cannulated rats, it was estimated that 3.69% of the administered dose of lycopene was absorbed systemically (i.e. $F_{total} = F_{lymph}$ (3.4%) + F_{portal} (0.29%)). The finding that F_{total} in lymph-cannulated rats (i.e. 3.69%) was greater than the estimate of overall bioavailability in non-lymph-cannulated rats (i.e. 1.84%), was similar to that reported for other highly lipophilic drug compounds.[36,37] This may therefore indicate either pre-systemic clearance of lycopene within the lymphatics or an altered systemic clearance pattern for lymphatically transported lycopene.

LCFA mixed micelles promoted the intestinal lymphatic uptake of lycopene to a significantly greater extent than MCFA mixed micelles. It is generally believed that drug uptake via the intestinal lymphatics is strongly associated with the uptake of fatty acids, synthesis of TRLS within the enterocyte and subsequent secretion into lymph lacteals. Therefore, formulations that stimulate TRL turnover within the enterocyte have been reported to increase the intestinal lymphatic transport of drugs.[38,39] The higher extent of lymphatic transport of lycopene for LCFA mixed micelles most likely reflects the greater impact of LCFA on stimulating TRL synthesis within enterocytes. Similarly, Caliph *et al*.[37] reported that the lymphatic transport of the lipophilic antimalarial halofantrine was highly dependent on the chain length of the co-administered triglyceride lipid and increased with increasing chain length. In summary therefore, LCFAs are more effective at enhancing intestinal lymphatic transport of lycopene than MCFAs.

Interestingly, the FaSSIF and FeSSIF formulations resulted in greater extents of intestinal lymphatic transport of lycopene compared with the bile salt mixed micelle vehicles (Table 2). This observation was unexpected given that a number of studies have reported that formulations that closely resemble the end products of lipid digestion, such as mixed micelles of fatty acids, are preferred, in terms of maximal intestinal lymphatic drug transport.[40,41] In addition, the higher extent of lycopene transport from FaSSIF/FeSSIF cannot be explained by an increase in TRL synthesis in enterocytes, as the extent of triglyceride turnover was actually lower for the FaSSIF/FeSSIF formulations compared with the mixed micelle systems (Table 2). Our findings therefore suggest that the physiological buffer media may be more efficient than fatty acid–bile salt mixed micelles at promoting lymphatic transport of lycopene. Possible reasons for this were examined further by evaluating the loading of lycopene in intestinal triglyceride. The cumulative percent dose of lycopene transported as a function of cumulative mass of triglyceride collected in intestinal lymph over 8 h is presented in Figure 4. A strong positive correlation was observed for all formulations. This indicates that lycopene transported in intestinal lymph is strongly associated with triglyceride turnover in intestinal cells. In the case of the LCFA and MCFA mixed micelle formulations, the slopes of the lines describing these relationships were similar and suggest therefore that the apparent loading or content of lycopene in intestinal triglyceride was similar in both. However, whereas the actual drug loading or efficiency of drug transfer to intestinal triglyceride is similar for both mixed micellar formulations, critically it is the ability of the LCFA mixed micelles to stimulate a greater extent of triglyceride turnover (i.e. 38.21 vs 16.52 mg) that results in a significantly higher overall lymphatic transport of lycopene.

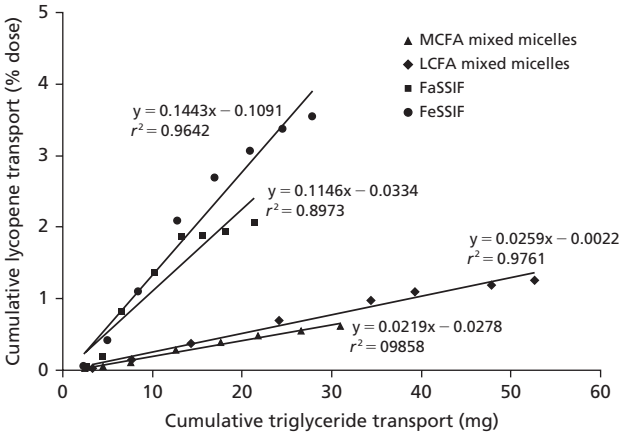

Figure 4 Correlation between cumulative mass of triglycerides and cumulative % dose of lycopene transported in rat lymph. Lycopene was administered intraduodenally solubilised in a range of biorelevant media: MCFA mixed micelles; LCFA mixed micelles; FaSSIF; and FeSSIF

In the case of FaSSIF/FeSSIF preparations, the slopes of the trend lines obtained were higher, reflecting a higher drug loading in intestinal lymph, compared with those observed for the mixed micelle systems. Administration of FaSSIF/FeSSIF therefore results in the highest amount of drug transfer into the lymph, despite stimulating the lowest amount of total triglyceride transport, compared with the mixed micelle systems (Table 2). In other words, while there is still a strong correlation between drug uptake and total triglyceride uptake, it appears that drug transfer into the intestinal lymph is more efficient following administration in FaSSIF/FeSSIF. Given that the total amount of lipid present in the FaSSIF/FeSSIF formulation is low (i.e. 0.75–3.75 mM lecithin, equivalent to 0.5–2 mg/ml of LCFA), it can be assumed that the majority of lipids collected in the lymph following administration of FaSSIF/FeSSIF will be derived from endogenous sources. This finding is similar to that of a previous study wherein the highest loading in intestinal lymph of the lipophilic drug saquinavir was found for a microemulsion formulation, which similarly resulted in a relatively small increase in lymph triglyceride transport.[39] It can therefore be postulated that the greater efficiency of drug uptake in the intestinal lymphatics may reflect a greater ability of 'endogenous' lipids to transport drug lymphatically. In support of this, a recent study involving a mechanistic evaluation of the factors influencing intestinal lymphatic drug transport has shown that the manner in which endogenous and exogenous lipids are trafficked through the lymph lipid precursor pool (LLPP) are different.[42] This in turn has been shown to influence the degree and extent of intestinal lymphatic drug transport. The LLPP is a pool of intracellular lipids within the enterocytes that is derived from both exogenous and endogenous lipids. Formulations that have a high lipid content, such as the LCFA mixed micelles (i.e. 20 mg/h), will expand or swell the LLPP, primarily due to exogenously lipid present in the formulation. This results in increased triglyceride turnover and stimulation of drug transport via the intestinal lymphatic route, similar to that observed for the LCFA bile salt mixed micelles. However, in the case of low lipid dose formulations, such as in the case of FaSSIF/FeSSIF, the LLPP will consist primarily of endogenously derived lipids, which may be either biliary derived or non-biliary derived. Formulation components that stimulate biliary derived endogenous lipids were found to promote lymphatic transport of the highly lipophilic drug halofantrine to a greater extent than non-biliary derived lipids. Lysophosphatidyl choline (LPC), the digestion product of phosphatidyl choline, was identified as a component that stimulates biliary derived lipids in the LLPP, and resulted in significant increases in intestinal lymphatic transport of halofantrine.[42] Lecithin, a phospholipid, is the primary lipid component in both FaSSIF and FeSSIF. Hence this hypothesis may be used to explain the higher loading of lycopene in intestinal triglyceride and also the greater overall extent of lymphatic transport of lycopene in FaSSIF and FeSSIF. Therefore, in terms of optimising oral absorption of lycopene, a formulation rich in phosphatidyl choline or its derivatives, which appear to stimulate or swell the endogenous lipid pool, may promote higher loadings and therein a greater overall extent of lymphatic transport of lycopene.

Conclusions

For highly lipophilic compounds, such as lycopene, there are a number of critical steps influencing the absorption process, including the solubility in the physiological media, the uptake into intestinal cells and the subsequent release into general circulation. Solubility of lycopene is higher in lipid-rich physiological media and therefore lipid-based formulations may be a suitable formulation approach for enhancing lycopene bioavailability. The absolute bioavailability of lycopene after 8 h was estimated to be 1.84 ± 0.39% following intraduodenal administration of lycopene solubilised in FeSSIF to anaesthetised rats. The intestinal lymphatic transport route is the major uptake mechanism of lycopene from the gastrointestinal tract, accounting for in excess of 84% of all the lycopene absorbed systemically. The extent of intestinal lymphatic transport of lycopene in the rat ranged from 0.6 to 3.4% of administered dose, with the highest extent of transport observed under simulated fed-state conditions. Intestinal lymphatic transport of lycopene is highly correlated with the extent of intestinal lymphatic transport of intestinal triglyceride, confirming that lycopene transport in the lymph is associated with the triglyceride-rich lipoproteins. Long-chain lipids or formulation components such as lecithin are favourable in terms of maximising intestinal lymphatic transport and therein overall absorption of lycopene.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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