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Plasma profiles of lycopene after single oral and intravenous administrations in dogs

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Abstract

The objectives of this study were to identify the factors limiting the absorption of purified lycopene after oral administration, and to comparatively assess plasma data sets after single oral and intravenous administrations in dogs to define the conditions for performing an absolute bioavailability study. Solubility of purified lycopene (all-trans, 93.5%) was determined in media simulating the conditions in the fasted and in the fed upper gastrointestinal lumen. After evaluating the plasma levels achieved following single administrations of purified lycopene powder to fasted and fed dogs at escalating doses (75–750 mg), a crossover study was performed in four fed female mongrel dogs at two phases. In phase I, one soft gelatine capsule (10 mg lycopene) with 500 mL milk was administered orally. In phase II, 500 mL milk was administered orally and 250 mL 5% dextrose containing 5 mg lycopene in the form of a binary system with hydroxypropyl-β-cyclodextrin (HP-β-CyD) was administered intravenously over 3.5 h. In-vitro and preliminary canine studies confirmed that, after oral administration of lycopene in solid form, arrival of lycopene into the systemic circulation was limited by lymphatic transport and, in addition, if the administered dose was higher than approximately 2 mg, by intralumenal solubility. During the first 50 h after single administrations to fed dogs, lycopene plasma levels were lower after intravenous than after oral administration. This could have been related to capacity limited elimination of lycopene and/or route-dependent disposition kinetics. Estimation of the amount of lycopene reaching the systemic circulation after oral and after intravenous administration requires separate estimations of total body clearance of lycopene.

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Introduction

Lycopene, the predominant carotenoid in tomatoes and tomato products (Clinton 1998) is a highly lipophilic antioxidant (clogP 17.6, http://www.syrres.com/esc/est_kowdemo.htm, accessed 20 October 2005) that is thought to be associated with a reduced risk for prostate cancer (Giovanucci 1999).

To date, there have been various studies in which the kinetics of lycopene in the plasma of dogs (Korytko et al 2003) and of man (Stahl & Sies 1992; Johnson et al 1997; Diwadkar-Navsariwala et al 2003; Brown et al 2004; Gustin et al 2004) have been studied after single oral administrations of crude lycopene products. With respect to the quantification of the amount of lycopene reaching the systemic circulation, all of those studies suffered from the absence of intravenous data. Another drawback of some of those studies was their parallel designs; given the high inter-subject variability, analysis of dose–response data becomes difficult (Diwadkar-Navsariwala et al 2003; Korytko et al 2003). Finally, absorption studies in man are complicated by the presence of endogenous lycopene stored in various tissues. Baseline levels in man make the study of the absorption phase problematic e.g. increase of blood levels after single dose administration may not be significant, unless selective recruitment of subjects is done (e.g. Diwadkar-Navsariwala et al 2003) or deuterated lycopene is administered and advanced analytical techniques are used (Tang et al 2005).

The objectives of this study were two-fold. The first was to identify the factors limiting the absorption of purified lycopene after oral administration. The second was to comparatively assess plasma data sets after single oral and intravenous administrations using the canine model and, as much as possible, similar doses. Intravenous administration has become possible due to a recently developed formulation in which lycopene has been solubilized by hydroxypropyl-β-cyclodextrin (HP-β-CyD) (Vertzoni et al 2006). Due to its relevance with

the human gastrointestinal system (e.g. Kararli 1995), the canine model is an appropriate in-vivo model for assessing the oral absorption characteristics of actual dosage forms of non-ionizable, lymphatically transported compounds (Kararli 1995; Khoo et al 2001). For lycopene specifically, the basic pharmacokinetic parameters in dogs seem to be comparable with that in man (Korytko et al 2003). Finally, due to the lack of lycopene reserves (absence of baseline plasma levels) in dogs, data variability should be lower. Therefore, results from this study should provide the basis for performing absolute bioavailability studies of dosage forms of lycopene in man.

Materials and Methods

Dosage forms

All-trans lycopene was isolated from tomato paste and it was kindly donated by the Laboratory of Pharmacognosy and Natural Products, National and Kapodistrian University of Athens, Athens, Greece (purity 93.5%; Vertzoni et al (2006)). Soft gelatin capsules contained 10 mg lycopene (labelled amount) in a triglyceride solution (Lot# 498511 2316, Herbal Select, Ontario, Canada). The intravenously administered solution consisted of 250 mL 5% glucose solution containing 5 mg all-trans lycopene complexed with hydroxypropyl-β-cyclodextrin (HP-β-CyD), so that the concentration of HP-β-CyD in the administered solution was 5 mg mL^{-1} (Vertzoni et al 2006).

In-vitro experiments

Dissolution studies of lycopene powder (750 mg (1.4 mmol)) were run in triplicate at 37 ± 0.5°C using USP II Apparatus (Distek dissolution tester, model 2100B, North Brunswick, NJ) and with the paddle rotating at 100 rev min^{-1} in 500 mL dissolution medium. Experiments were performed until plateau was reached, in fasted state simulating gastric fluid (FaSSGF (Vertzoni et al 2005a)), in fasted state simulating intestinal fluid (FaSSIF (Galia et al 1998)), in long-life cow's milk (Landesgenossenschaft Ennstal Molkereibetriebe, Steinach, Austria) with 3.5% fat content (mainly triglycerides (21% oleic (18:1), 23% palmitic (16:0), 11% myristic (14;0), 10% stearic (18:0)) (Walstra & Jenness 1984)) to simulate fed gastric conditions (Macheras et al 1986; Galia et al 1998), and in fed state simulating intestinal fluid (Galia et al 1998) that additionally contained 5 mM glycerol monooleate ($FeSSIF_{GMO}$) to simulate the presence of lipolytic products in the aqueous phase of duodenal contents (Armand et al 1996).

Canine studies

Canine studies were performed at an animal facility operating according to European Union regulations for the maintenance and experimentation on animals, and which had been approved by the Veterinary Directorate of the Municipality of Athens.

Four female mongrel dogs (27–32 kg, 3–4-years old) participated in the study. Dogs were fasted for 16 h, but with water available, before each administration.

During the first 12 h after administration of lycopene, blood samples (6 mL per sample) were drawn by means of an indwelling catheter positioned in a suitable foreleg vein. Subsequent samples were drawn by individual venipuncture. After centrifugation, plasma was stored at −20°C until assayed. Six hours after the oral administration and six hours after the beginning of intravenous administration (see later), each dog consumed 200 mL tap water and 150 g non-lycopene containing pellets (18% triglycerides (40% oleic (18:1), 21% linoleic (18:2), 22% palmitic (16:0)), Nutro Products Inc., CA) within 15 min. At 10, 24, 30, and 48 h following the oral administration of lycopene and the initiation of the intravenous administration all four dogs were offered the same type and amount of food. At these times the meal was completely consumed by all four dogs.

The wash-out period between phases was at least three weeks for each dog.

Relevant protocols were approved by the Committee for Research of the National & Kapodistrian University of Athens.

Evaluation of plasma levels after single administration of lycopene powder

To avoid the interference of excipients with the absorption process, this study was performed using only lycopene powder. This study had four phases and was performed using dog 1 and dog 2. On four different occasions each dog was administered via an orogastric tube: 75 mg lycopene suspended in 250 mL water; 300 mg lycopene suspended in 250 mL water; 750 mg lycopene suspended in 500 mL water; or 750 mg lycopene suspended in 500 mL long-life cow's milk (3.5% fat, Landesgenossenschaft Ennstal Molkereibetriebe, Steinach, Austria).

Since the objective of these sets of experiments was to evaluate the characteristics of lycopene arrival into the general circulation, samples were drawn for up to 48 h post-dosing.

Comparative assessment of lycopene plasma levels after single oral and intravenous administrations

This study had two phases and it was performed on a cross-over basis using dog 1, dog 2, dog 3, and dog 4. To be able to administer similar oral and intravenous doses (so that a meaningful comparison of the resulting data sets could be made) the oral administration had to be performed under conditions that overcame barriers to reach the systemic circulation as much as possible. Therefore, in the first phase, each dog was administered via an orogastric tube one soft gelatin capsule containing 10 mg lycopene with 500 mL long-life cow's milk (3.5% fat, Landesgenossenschaft Ennstal Molkereibetriebe, Steinach, Austria). In the second phase, each dog was administered 500 mL long-life cow's milk via an orogastric tube and 250 mL 5% dextrose solution containing 5 mg lycopene complexed with HP-β-CyD (Vertzoni et al 2006) intravenously over 3.5 h (infusion rate 1.2 mL min^{-1}).

A few minutes before the onset of each phase, a blood sample was drawn to measure lycopene, total cholesterol and triglyceride levels. After administration, and to assess if lycopene reached the general circulation with chylomicrons, lycopene and triglyceride levels were measured in plasma. Based on data from the investigative set of experiments (the evaluation of plasma levels after single administration of lycopene powder), the low doses administered in this study, and ethical considerations (dense sampling injured the veins of the dogs), blood samples in this study were drawn for up to 56 h post-milk administration.

Analysis of samples

In the in-vitro studies and canine studies after administration of lycopene powder, lycopene was assayed using a validated previously published HPLC-UV method (Tzouganaki et al 2002). Application of this method resulted in a limit of quantification of 78 nM.

For the determination of lycopene levels in plasma after soft gel capsule and lycopene-HP-β-CyD administrations, a more sensitive analytical method recently developed and validated in our laboratory was used (Vertzoni et al 2005b). This method had a limit of quantification of 7.5 nM (Vertzoni et al 2005b).

Total cholesterol and triglycerides were measured by a Biotecnica BT1000 autoanalyser (Biotecnica Instruments S.p.A., Rome, Italy) with test kits from Menarini (A. Menarini Industrie Farmaceutiche Riunite S.r.L., Diagnostics Division, Firenze, Italy). The test sensitivity in terms of detection limit was 4 mg dL^{-1} (0.103 mmol L^{-1}), and 1 mg dL^{-1} (0.01 mmol L^{-1}) for total cholesterol and triglycerides, respectively.

Data treatment

Maximum concentrations of lycopene in various biorelevant media were compared with one-way analysis of variance accompanied by the Tukey post-hoc test.

The area under every individual lycopene concentration vs time profile up to 48 h post-dosing ($AUC_{0-48\,h}$) was estimated using the linear trapezoidal rule. Comparison of $AUC_{0-48\,h}$ data after oral administration with $AUC_{0-48\,h}$ data after intravenous administration (after taking into account the dose difference) was performed with a paired *t*-test.

All statistical tests were performed with Sigmastat for Windows (version 2.03, SPSS Inc.) and significance was considered at the 0.05 level.

Compartmental modelling of individual intravenous data and of weighted (by the standard deviation) individual intravenous data was applied using WinNonlin Professional (version 3.1, Pharsight Corporation).

Results

In-vitro data

Plateau levels in Figure 1 reflected the equilibrium solubility of lycopene in the relevant media. Solubility was minimal in media simulating the fasting intralumenal conditions (< 26 nM in FaSSGF and approximately 85.5 nM in FaSSIF). As expected from the high lipophilicity of lycopene, solubility in fed state simulating media was significantly increased ($P < 0.001$) to approximately 1902 nM in milk and 1378 nM in $FeSSIF_{GMO}$ (Figure 1), but it was still negligible compared with the plateau level that would be achieved if the entire dose was dissolved in the dissolution medium (2800 μM).

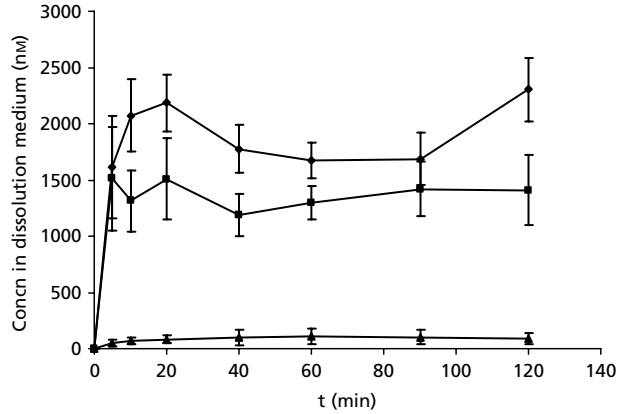

Figure 1 Cumulative in-vitro dissolution data of lycopene powder in milk (♦), FaSSIF (▲), and $FeSSIF_{GMO}$ (■). Lycopene concentration in FaSSGF was less than 26 nM at all time points. The theoretical concentration in the vessel if the entire dose (750 mg = 1.4 mmol) was totally dissolved in the 500 mL of the dissolution medium was 2800 μM. Values are mean ± s.d.

Evaluation of plasma levels after single administration of all-trans lycopene powder

A few minutes before any administration in this study, the concentration of lycopene in plasma was lower than 26 nM (i.e. lower than the detection limit of the analytical method applied in this set of studies (Tzouganaki et al 2002)).

A dose of 75 mg administered in the fasting state resulted in concentrations lower than the limit of quantification of the analytical method (<78 nM) during the first 48 h post-dosing. A dose of 300 mg administered again in the fasted state resulted in quantifiable concentrations only at times 24 (119.2 nM) and 48 h (316.6 nM) post-dosing.

After administration of 750 mg in two dogs (corresponding to 23.2 and 27.4 mg kg^{-1}) in the fasting and in the fed states, plasma concentrations (up to approximately 2000 nM, data not shown) were highly variable but of the same order of magnitude with the values observed in a previous canine study (Korytko et al 2003), where similar doses of a crude lycopene product (starch particles containing 5% lycopene) had been administered. Immediately after lycopene administration and for as long as the gastrointestinal tract was under fasting conditions, practically no lycopene arrived in the general circulation. Onset of lycopene concentration in plasma was observed ~2 h after administration of a meal. Plasma levels continued to rise at times longer than 10 h after administration i.e. after removal of lycopene from the small intestine and/or from the canine colon (e.g. De Zwart et al 1999) where

absorption might have also been possible (Oshima et al 1999). Maximum plasma concentrations were observed at least 24 h post-dosing. Therefore, the $AUC_{0-48\,h}$ data corresponded to partial areas reflecting the rates of appearance (and not the total amount) of lycopene in plasma (e.g. Macheras et al 1994). Administration in the fed state led to increased AUC_{0-48h}^{powder} by 204% and 144% in dog 1 and dog 2, respectively. The slow disposition of lycopene observed in this study (data not shown) and in the previous canine study (Korytko et al 2003) were in agreement with the slow disposition in man (e.g. Diwadkar-Navsariwala et al 2003).

Comparative assessment of lycopene plasma levels after single oral and intravenous administrations

A few minutes before any administration, lycopene concentration in plasma was measured to be lower than the detection limit of the analytical method used in this set of experiments (Vertzoni et al 2005b) i.e. less than 2.5 nM. The mean ± s.d. total cholesterol levels before the oral and the intravenous administrations of lycopene were 4.1 ± 1.4 and 3.3 ± 0.9 mM, respectively. Corresponding triglyceride levels were 0.44 ± 0.17 and 0.41 ± 0.05 mM, respectively. These values were similar to previously reported values in healthy fasted dogs (Goldstein et al 1980; Yasunobu et al 1997; Chikamune et al 1998; Bailhache et al 2004).

Figure 2 shows the individual lycopene and triglyceride profiles in plasma after oral administration of one soft gelatin capsule of lycopene with milk. In all four dogs, the lag time before the onset of plasma levels of lycopene was identical to the lag time for the onset of increase of triglycerides in plasma (~2 h). Also, in all four dogs, plasma lycopene levels increased with plasma triglycerides levels for times up to 6–10 h post-dosing. At later times the correlation of lycopene and triglycerides levels was sustained only for dog 3 and dog 4. The uncontrolled duration of meal consumption at later times could be one reason for the lack of correlation in the other two dogs.

Figure 3 shows the individual lycopene and triglyceride plasma profiles after intravenous administration of lycopene and oral administration of milk. As in Figure 2, triglyceride levels did not rise significantly during the first 2 h after milk administration. Despite the constant infusion rate, at times when triglyceride levels started to increase, lycopene levels also showed a sharp increase. However, unlike in Figure 2, the rise of triglyceride levels lasted for a limited period of time; triglyceride levels declined sharply 3 h post-milk administration to reach almost baseline levels at 6 h post-dosing. The sharp

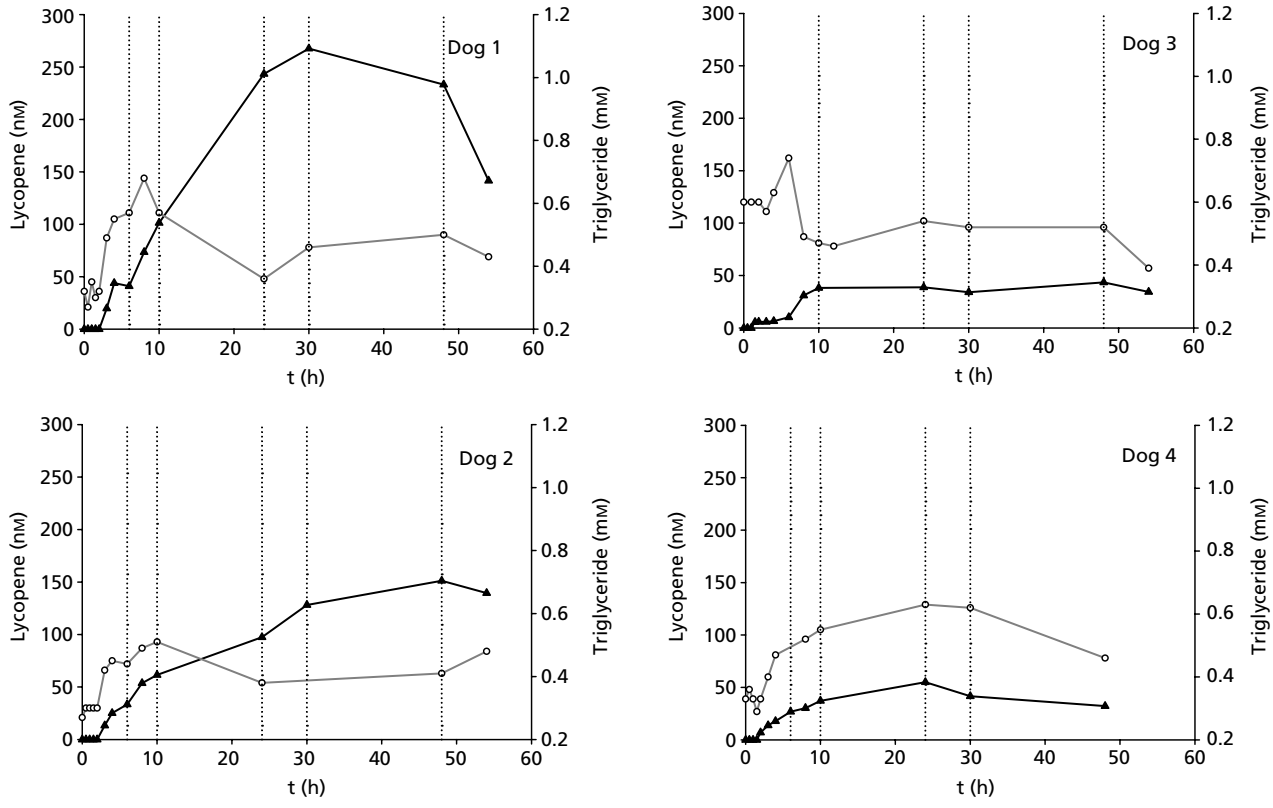

Figure 2 Individual plasma lycopene (▲) and triglyceride (○) levels after single oral administration of one soft gelatine capsule containing 10 mg lycopene with 500 mL milk to four fasted female dogs. Dotted lines indicate the times when the dogs started to consume 150 g of pellets and 250 mL water. At 6 h, dog 3 denied eating whereas the rest of the dogs consumed the meal within 15 min.

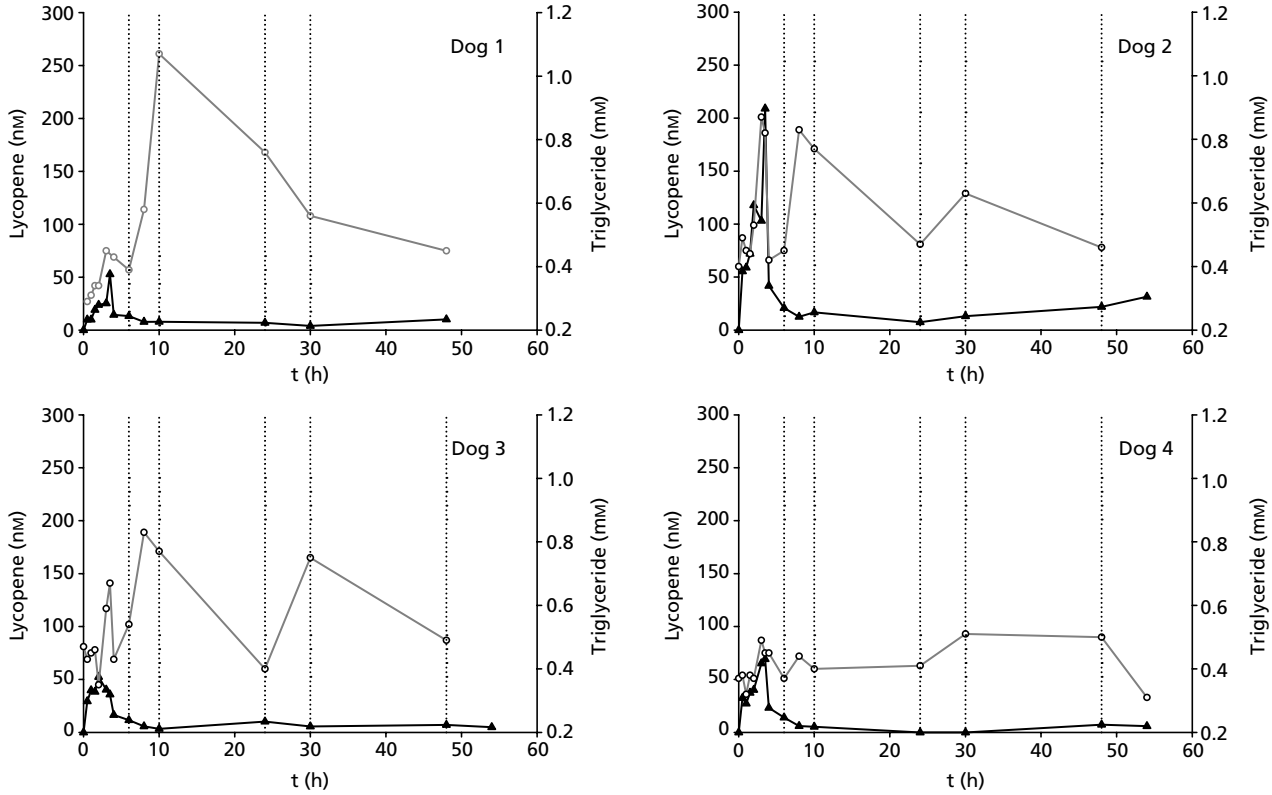

Figure 3 Individual plasma lycopene (▲) and triglyceride (○) levels after single intravenous administration of 250 mL lycopene-HP-β-CyD solution containing 5 mg lycopene accompanied by single oral administration of 500 mL milk to four fasted female dogs. Dotted lines indicate the times when the dogs started to consume 150 g of pellets and 250 mL water. At 6 h, consumption was complete within 15 min.

decline of triglyceride levels between 3 and 6 h post-dosing was accompanied by an analogous sharp decline of lycopene levels. Finally, as with some dogs in Figure 2, at times longer than approximately 6 h post-milk administration, lycopene levels did not correlate with triglyceride levels. The small increase of lycopene levels during the first 2 h after the initiation of intravenous infusion (i.e. when triglyceride levels were minimal) and the sharp decline of lycopene levels with triglyceride levels immediately after completion of the infusion led to plasma levels much lower than after the administration of soft gelatin capsules; individual values of the ratio

$$\frac{(AUC_{0-48h}^{capsule})(Dose_{milk}^{iv,5})}{(AUC_{0-48h}^{iv})(Dose_{milk}^{capsule,10})}$$ were higher than 1 for every dog

and ranged from 1.8 to 10.8. However, the difference between $AUC_{0-48h}^{capsule}$ and AUC_{0-48h}^{iv} (after taking into account the dose difference) was not statistically significant ($P = 0.165$) due to the low power of the experimental design ($1-\beta = 0.191$). It was noteworthy that lycopene levels started to increase again at late time post-infusion (24–30 h after the initiation of the infusion depending on the dog, Figure 3), suggesting that the rapid disappearance from plasma after intravenous administration was, at least partly, due to reversible distribution at peripheral tissues. It should be mentioned that fitting a compartmental model to the unweighted and weighted intravenous lycopene data collected in this study was not successful, due to the increasing plasma levels at times near the end of the sampling period. Fitting to the intravenous data was unreliable even when the late time points (at which plasma levels slightly increased) were not considered for the fitting procedure.

Discussion

Assuming that the total volume of luminal contents available for dissolving solid lycopene during its residence in the human fed small intestine will be at most 2 L (Dressman et al 1998), the solubility data in $FeSSIF_{GMO}$ (Figure 1) suggested that the maximum amount of lycopene that would be dissolved during upper gastrointestinal residence was up to 4 μmol i.e. approximately 2 mg. Therefore, solid doses of lycopene higher than approximately 2 mg should show solubility limited disappearance characteristics from human contents in the fed state. These observations were in agreement with the non-linear increase in gastrointestinal absorption of lycopene with the dose in fed volunteers (Gustin et al 2004). In dogs, bile salt levels in the fed small intestine were higher (Kalantzi et al 2006). Therefore, the lowest dose limit for solubility-limited disappearance from the luminal contents should be somewhat higher in dogs. The non-linear increase of total exposure values in dogs has indeed been shown (Korytko et al 2003), but the

parallel design of that study and the high inter-dog variability does not allow for further discussion. In this study, the value of $\frac{(AUC_{0-48h}^{capsule})(Dose_{milk}^{powder,750})}{(AUC_{0-48h}^{powder})(Dose_{milk}^{capsule,10})}$ was 12.96 and 13.90 for dog 1 and dog 2, respectively, confirming that the rates of appearance into the systemic circulation were much higher when the dose was administered as solution in triglycerides.

Apart from being solubility limited, the arrival of lycopene in plasma after oral administration was additionally limited by lymphatic transport and the lag time for the onset of appearance into the systemic circulation after single administration with food was approximately 2 h (Figure 2). Similar lymphatic transport characteristics have been observed in man (Clevidence & Bieri 1993; Diwadkar-Navsariwala et al 2003).

The rise of lycopene plasma levels at times longer than 10 h after oral administration (Figure 2) could be related to capacity limited elimination characteristics, to distribution of lycopene into tissues that turn it over to the systemic circulation (as is the case in man (Diwadkar-Navsariwala et al 2003)), or to the entrapment of a significant amount of lycopene into lipophilic regions of the intestinal mucosa (e.g. the cell membrane) from which it is removed only if substantial amounts of lipids are also present.

The low plasma concentrations (compared with oral data) during the first 50 h after intravenous administration could be due to capacity limited elimination characteristics (the oral dose was double that of the intravenous dose), to route-dependent distribution kinetics, or to the vehicle used for the intravenous administration.

Assessment of the effect of HP-β-CyD on lycopene plasma levels requires prior estimation of the stability constant of the binary lycopene-HP-β-CyD system. This is extremely difficult to make due to the very low aqueous solubility of lycopene (Vertzoni et al 2006). According to Frijlink et al (1991a), the disposition characteristics of flurbiprofen (clogP 3.81) after intravenous administration with HP-β-CyD (concentration of HP-β-CyD in the formulation: 70 mg mL^{-1}) in rats were modified by the presence of HP-β-CyD. The plasma concentrations were (in contrast to this study) higher for a limited period of time (approximately half an hour) after completion of administration. In another study, the disposition characteristics of the more lipophilic itraconazole (clogP 6.16), administered intravenously with HP-β-CyD (concentration of HP-β-CyD in the formulation: 400 mg mL^{-1}) in man, were not affected by the presence of HP-β-CyD (Barone et al 1998; Willems et al 2001; Zhao et al 2001). Further, it is known that the stability constant of cholesterol with HP-β-CyD is high (Frijlink et al 1991b) and in this study cholesterol levels were much higher than that of lycopene i.e. lycopene should be easily displaced by cholesterol from the cyclodextrin cavity. Therefore, based on literature data, HP-β-CyD should not have affected substantially the disposition characteristics of lycopene.

Based on the small rise of plasma levels 24–30 h after the initiation of the infusion (Figure 3), and the existence of fast and slow turnover lycopene compartments in the human body (Diwadkar-Navsariwala et al 2003), the possibility that co-processing of chylomicrons with lycopene, after oral administration, led to longer plasma residence of both triglycerides and lycopene than after intravenous administration of lycopene in the fed state, appeared likely. A similar observation was made with halofantrine (clogP ~8) for which the post-prandial absolute bioavailability in dogs was estimated to be higher than 100% (Humberstone et al 1996, 1998). However, the mode of administration in those studies did not allow for observing possible route-dependent disposition characteristics in the fed state. It would be interesting to know if the route-dependent distribution kinetics of lycopene had an impact on total body clearance. Estimation of total body clearance of lycopene should involve extensive sampling for several days and, for ethical reasons, sampling-time intervals at early times should be wider than the ones applied in this study.

Conclusion

After oral administration, lycopene arrived in plasma with chylomicrons and estimation of the amount reaching the systemic circulation should be assessed in the fed state. During the first 50 h after single administrations in fed dogs, lycopene plasma levels were lower after intravenous than after oral administration. This could have been due to capacity limited elimination of lycopene and/or route-dependent disposition kinetics. Therefore, estimation of the amount of lycopene reaching the systemic circulation after oral and after intravenous administration requires separate estimations of total body clearance of lycopene.

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