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Author(s)	Sato, Yuki; Joumura, Tatsuru; Nashimoto, Shunsuke; Yokoyama, Sayaka; Takekuma, Yoh; Yoshida, Hideto; Sugawara, Mitsuru
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Enhancement of lymphatic transport of lutein by oral administration of a solid dispersion and a self-microemulsifying drug delivery system

Yuki Sato ^{a, †}, Tatsuru Joumura ^{a, †}, Shunsuke Nashimoto ^a, Sayaka Yokoyama ^a, Yoh Takekuma ^a, Hideto Yoshida ^b, Mitsuru Sugawara ^{a, *}

^a Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

^b R&D Division, Kewpie Corporation

2-5-7, Sengawa-cho, Chofu-shi, Tokyo 182-0002, Japan

[†] Equally contributed to this work

* To whom correspondence should be addressed

Mitsuru Sugawara, Ph. D.,

Laboratory of Pharmacokinetics, Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

Tel/Fax: +81-11-706-3923/ +81-11-706-4984

E-mail: msuga@pharm.hokudai.ac.jp

Abstract

Lutein is located in the macula lutea in the human eye. Since humans cannot synthesize lutein de novo, it must be digested as food. Some studies including our previous study showed very low absorption of lutein after oral administration. Those studies also suggested that the absorption route of lutein from the small intestine involves not only the blood but also the lymph. The aim of this study was to clarify the transfer of lutein into lymph and the tissue distribution after oral administration of a solid dispersion (SD) and a self-microemulsifying drug delivery system (SMEDDS) for improvement of the absorption. We used thoracic lymph-cannulated rats. It was shown that the plasma concentrations of lutein in the SD and SMEDDS groups were increased compared with that in the powder group. The absorption of lutein after oral administration of each formulation was clearly evaluated by its cumulative amount in lymph. Our data clearly showed that lutein is transferred into the lymph stream from the small intestine.

Keywords: lutein (PubChem CID: 5281243); lymph; absorption; solid dispersion; self-microemulsifying drug delivery system; intestine.

1. Introduction

Lutein is a major carotenoid that is present in dark green leafy vegetables such as spinach and kale and in various fruits [1]. In human eyes, lutein is a macular pigment that is located in the macula lutea, yellow spots, between incoming photons and photoreceptors [2]. Lutein has been thought to provide protection to the photoreceptors as blue light filters and powerful antioxidants [3]. It has been reported that a high serum carotenoid level and high dietary intake of lutein are associated with lower relative risk of age-related macular degeneration (AMD) [4,5]. AMD is a leading cause of irreversible blindness in the elderly in developed countries [6]. One of the effective treatments for AMD is intravitreal injection of an anti-vascular endothelial growth factor (VEGF) drug. However, this treatment is stressful for AMD patients. It is important to prevent AMD in daily life. It will take a long time to establish an easier treatment, although a new treatment using induced pluripotent stem (iPS) cells has been reported in Japan [7].

We previously reported that the bioavailability of lutein was about 5% [8]. Various pharmaceuticals for improvement of this low absorption of lutein have been reported [9-11]. A self-emulsifying phospholipid suspension and solid nanoparticles would be effective for improvement of the intestinal absorption of lutein. The values of T_{\max} from plasma concentration data differed greatly in previous studies (2-12 h), though the formulation and

dose of lutein were different. We consider that the important step of the absorption is dissolution of lutein from each formulation and reformation of micelles in the intestine. In the case of absorption of dietary lipids, chylomicrons would be formed in epithelial cells and transferred to the lymph stream [12,13]. It is possible that the transport of lutein via the lymphatic route causes a low plasma concentration of lutein. However, there has been no definite report on the transport of lutein into the lymph stream after oral administration.

In this study, we focused on the transfer of lutein into the lymph stream and its tissue distribution after oral administration of a solid dispersion (SD) and a self-microemulsifying drug delivery system (SMEDDS), which are effective for improvement of its absorption. We performed thoracic lymph cannulation and investigated the lymph concentration and tissue distribution of lutein.

2. Materials and Methods

2.1. Chemicals and reagents

Lutein (MW 568.97, PubChem CID: 5281243) (85% powder) was kindly donated by FANCL Corp. (Kanagawa, Japan). Reagents were purchased from Wako Pure Chemical Industries, Ltd., (Osaka, Japan) unless otherwise noted. All reagents were of the highest grade

available and used without further purification.

2.2. Animals

Male Wistar rats, aged 5 or 6 weeks (160-180 g in weight), were obtained from Jla (Tokyo, Japan). All rats were housed in plastic cages (270 mm × 440 mm × 187 mm, Natsume Seisakusyo Co., Ltd., Tokyo, Japan). There were 3-4 rats in each cage. The housing conditions were the same as those described previously [14]. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”.

2.3. Preparation of a solid dispersion and a self-emulsifying drug delivery system

METOLOSE[®] (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) was used to prepare a suspension of lutein as described previously [15]. For preparation of a SD, polyvinylpyrrolidone (PVP) K-30 (GAF Chemicals Corp., KY, US) and Tween80 (polyoxyethylene (20) sorbitan monooleate) were used. The preparation method and composition of the SD were determined according to a previous study [16]. The final compositions of PVP K-30 and Tween80 in the SD containing 5% of lutein were 85% and

10%, respectively. The solid was dispersed in water (80 mg SD containing 4 mg of lutein per 10 mL of distilled water) because this solid itself could not be administered to rats. The dispersion was filtrated four times using a membrane filter (mixed cellulose ester type, pore size: 0.45 μm , 13 mm in diameter, ADVANTEC MFS Inc., CA, US) to obtain the solution. We confirmed that the concentration of lutein in this solution was 322.9 $\mu\text{g/mL}$ by an HPLC method described in 2.6. We administered this solution to rats as a SD considering the concentration and dose of lutein.

Egg yolk lysophosphatidylcholine LPC-1TM (Kewpie Corporation, Tokyo, Japan) was used for the preparation of an SMEDDS. The preparation method and ingredients of SMEDDS were determined with some modifications based on a previous study [17]. Four grams of soy bean oil was mixed with 142.9 mg of Tween20 (polyoxyethylene (20) sorbitan monolaurate) and then 75 mg of lutein was added to the mixture and the mixture was incubated at 60°C (TR-500H Pasolina hot stirrer, Iuchi Seieido Co., Ltd., Osaka, Japan). The solution was centrifuged (750 \times g for 10 min at room temperature) to obtain the supernatant (CF15RX, Hitachi-Koki Co., Ltd., Tokyo, Japan). Seventy-five microliters of filtered distilled water and 450 mg of concentrated glycerin were added to 75 mg of LPC-1TM and the mixture was stirred at about 60°C until a uniform consistency was obtained. The supernatant was added to the mixture drop by drop and the mixture was stirred until reaching a uniform consistency in order to obtain an SMEDDS in a gel form of lutein. We confirmed that the

concentration of lutein was 464.2 µg per 1 g gel by an HPLC method described in 2.6.

2.4. Measurement of particle sizes in the formulations

For confirmation of the properties of the SD and the SMEDDS prepared by the methods described in 2.3., the average particle sizes (z-average diameter) were measured by using a quasi-elastic light scattering method (Zeta Nano ZS; Malvern Instruments, Herrenberg, Germany) described previously [18].

2.5. Oral administration and collection of samples (plasma, tissue, lymph)

The rats were fasted for 14-16 h before the experiments. Thirty rats were used in all animal studies. Lutein was orally administered in powder (in 0.5% methylcellulose) or as the SD or SMEDDS. The dose of lutein was 2.5 mg/kg body weight in all groups.

Blood samples (about 300 µL) were collected and plasma samples were obtained as described previously [8]. Tissue samples (liver, spleen and kidney) were also excised at the designated time. The tissue samples were rapidly washed with saline and weighed and then homogenized with 1 mL distilled water /g tissue using a Potter-Elvehjem homogenizer with 20 strokes. For lymph collection, rats were anesthetized by an intraperitoneal injection (*i.p.*)

of sodium pentobarbital (50 mg/kg weight) prior to cannulation of the thoracic lymph duct. Briefly, a small midline incision was made in the abdomen. A cannula (SP tube; polyethylene tube, 0.5 mm in inner diameter [i.d] and 0.8 mm in outer diameter [o.d.], Natsume Seisakusyo Co., Ltd.) filled with heparin sodium (1,000 units/mL) was inserted into the thoracic lymph duct and secured within the abdominal cavity with a glue for tissue (Aron alpha A[®] “Sankyo”, Daiichi-Sankyo Company, Limited, Tokyo, Japan). After the operation, lutein was orally administered in an awake state and the rats were returned to their Bollman’s restrainer and given free access to distilled water during the lymph collection. Lymph was collected every 30 min for 0-9 h and every 60 min for 9-12 h after the administration. All samples were kept at -20°C until assay (biomedical freezer, SANYO Electric Co., Ltd., Osaka, Japan).

2.6. Analytical procedures

The conditions for extraction of lutein were the same as those described previously [8]. The concentration of lutein was determined in almost the same way as that in our previous study using an HPLC system equipped with an LC-20AD pump and an SPD-10AV UV-VIS detector (SHIMADZU, Kyoto, Japan) [8]. A mobile phase containing acetonitrile/ethyl acetate/distilled water (53/40/7, v/v/v) was used and the flow rate was 0.8 mL/min.

Tissues were weighed and tissue accumulation was calculated as g/tissue. Lymph concentration was determined and finally calculated as cumulative amount by multiplying by the sample volume.

2.7. Statistical analysis

Student's t-test was used to determine the significance of differences between two group means. Statistical significance among means of more than two groups was determined by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. Data are expressed as means with standard deviation (S.D.). Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Plasma concentration profile of lutein formulation after oral administration and particle sizes in the formulations

In the first part of this study, the plasma concentration of lutein was investigated up to 24 h after oral administration of each formulation. As expected, the absorption of lutein in the

powder (control) group was poor (Fig. 1-A). The plasma concentration of lutein in the SMEDDS group was slightly improved by 10-30 ng/mL in about 3-6 h compared to that in the control group (Fig. 1-B). There was a temporarily very high lutein concentration in the SD group (Fig. 1-C). However, the peak time of maximum concentration in each rat was not the same.

We evaluated the properties of our formulations to measure the particle size (z-average diameter) immediately after and at 3 days and 6 days after preparation at room temperature. The average particle sizes of the SMEDDS were 336.9 ± 93.6 nm and 349.6 ± 109.9 nm immediately after and 3 days after preparation, respectively. The SMEDDS particle size 6 days after preparation could not be determined due to phase separation. The average particle sizes of the SD were 154.3 ± 3.9 , 158.3 ± 12.3 and 181.8 ± 14.0 nm immediately after and 3 days and 6 days after preparation, respectively.

3.2. Tissue distribution of lutein formulation after oral administration

The tissue distribution of lutein was investigated 4 h after oral administration. The liver concentration of lutein in the SD group was much higher than that in the control group (Fig. 2-A). The liver concentration of lutein in the SMEDDS group was slightly higher than that in the control group. On the other hand, there were no significant differences between the

groups in kidney and spleen concentrations of lutein. We also investigated the lutein concentration in the intestinal mucosa 4 h after oral administration of each formulation. The concentration of lutein in the intestinal mucosa in the control group was low, whereas large amounts of lutein remained in the intestinal mucosa in the SMEDDS and SD groups (Fig. 2-B). These results suggested that lutein was also absorbed slowly 4 h after administration. We also confirmed that the concentration in each tissue after administration of only saline was not significantly different from that in the control (powder) group (data not shown).

We then investigated the tissue distribution of lutein 24 h after oral administration. Similar to the data obtained at 4 h after administration, it was found that lutein was distributed and accumulated in the liver in the SMEDDS and SD groups at 24 h after oral administration (Fig. 3-A). The concentrations of lutein in the kidney and spleen at 24 h after administration were not altered compared with those at 4 h after administration. It was also shown that the large amount of lutein was remained in the intestinal mucosa of SMEDDS and SD groups (Fig. 3-B). In particular, the large amount of lutein of SD group was shown in upper small intestine even though the plasma concentration was not altered from 12 h after administration of each formulation. These results suggested that lutein was largely distributed and accumulated in the liver with little accumulation in other tissues (kidney and spleen). These results also suggested that it is difficult to precisely evaluate the absorption of lutein by the plasma concentration profile, although lutein was absorbed.

3.3. Lymph concentration of lutein after oral administration of each formulation

We next performed thoracic lymph cannulation in rats and determined the lymph concentration of lutein up to 24 h after administration of each formulation. Since the flow rate of lymph is much slower than that of blood, each sample was cumulative. It was found that even the concentration of lutein in the control group was about 100 ng/mL about 9 h after administration (Fig. 4). The peak lutein concentration in the SMEDDS group (249.7 ± 120.5 ng/mL) was higher than that in the control group. The peak lutein concentration in the SD group (233.8 ± 163.9 ng/mL) was also higher than that in the control group and was similar to that in the SMEDDS group. These results suggested that differences of absorption of each formulation could be clearly evaluated by the lymph concentration of lutein.

The cumulative amount of lutein in lymph up to 24 h after administration of each formulation was calculated by multiplying the concentrations by each sample volume. The cumulative amount of lutein in the control group was 297.0 ± 100.4 ng (Fig. 5), and the cumulative amounts in the SMEDDS group and SD group were 1240.5 ± 503.9 ng and 1132.2 ± 230.6 ng, respectively. The cumulative amounts of lutein in lymph in the SMEDDS and SD groups up to 24 h were significantly larger than that in the control group.

4. Discussion

Absorption of food components and drugs from the gastrointestinal tract after oral administration is one of the important determinants of bioavailability, and the solubility of these components is thought to be a critical issue. Generally, poorly water-soluble components with poor membrane permeability classified into BCS (biopharmaceutics classification system) Class 4 show very low bioavailability [19,20]. Some pharmaceutical formulations are therefore needed to improve the absorption of such components. Lutein has received much attention for prevention of AMD due to its specific location in the eye and its powerful antioxidant activities. The solubility of lutein is very low because of its hydrophobicity and large molecular weight. We previously reported that the bioavailability of lutein was about 5% [8]. We also reported that some efflux transporters such as P-gp (P-glycoprotein) were not involved in the absorption of lutein [21]. It is possible that transport of lutein via the lymphatic route causes a low plasma concentration of lutein. However, there has been no definite report on transport of lutein into the lymph stream after oral administration. We therefore investigated the transfer of lutein into the lymph stream using thoracic lymph-cannulated rats. We prepared a SD and an SMEDDS and focused on the pharmacokinetic properties of lutein after oral administration of these formulations for

improvement of its absorption.

There were no differences in average sizes of the SD and SMEDDS between immediately after and 3 days after preparation. The properties of our SD and SMEDDS would be maintained for at least 3 days. We next investigated the plasma concentration profile of lutein after oral administrations of our formulations to rats. It was shown that the plasma concentration of lutein was slightly increased by administration of the SMEDDS, whereas there was little absorption of lutein after administration of the powder (Figs. 1-A, B). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the acceptable daily intake (ADI) for lutein and zeaxanthin is 0-2 mg/kg weight [22]. The dose used in the present study (2.5 mg/kg) did not greatly exceed the ADI. There have been some reports that the dose is much higher than the ADI, although some formulations showed improvement in the absorption of lutein [9]. The absorption of lutein was also improved by administration of our SD. However, it was shown that each plasma concentration of lutein rapidly increased and then decreased (Fig. 1-C). Some studies have shown great variations of plasma and tissue concentrations after oral administration of a pharmaceutical formulation [11]. We considered that the peak time and peak concentration of the data were too different individually to evaluate the absorption of lutein by the plasma concentration profile. We then investigated the tissue distributions (liver, kidney and spleen) of lutein after oral administration of our formulations. It was found that lutein distributed and accumulated

mostly in the liver, followed by the spleen and kidney (Fig. 2-A). This tendency is almost consistent with previous reports [8,23]. Compared with the amounts at 4 h after administration, the amounts of lutein were increased in all tissues at 24 h after administration (Fig. 3). The large amount of lutein of SD group was shown in upper small intestine 24 h after administration even though the plasma concentration was not altered from 12 h after administration of each formulation (Figs. 1, 3-B). These results suggested that orally administered lutein is slowly distributed to the gastrointestinal mucus layer and other tissues. Our results for the plasma concentration profile and tissue distribution showed that the absorption of lutein was improved after oral administration of the SMEDDS and SD. We consider that SMEDDS and SD was easy to mix bile and phospholipids and form mixed micelles in the intestinal lumen. In case of the administration of SMEDDS and SD, the solubility of lutein would be improved compared with that of the powder itself and would be easy to permeate intestinal epithelial cells.

We then focused on the transfer of lutein into the lymph stream. The lutein concentrations gradually increased from about 4-5 h and 1.5-3 h after administration of the SMEDDS and SD, respectively (Fig. 4). It was also shown that the lymph concentration of lutein was higher than the plasma concentration even in the powder group. Microemulsions are known to transfer into lymph, but we consider that lutein itself is likely to be transferred via lymphatic route, too. The very slow flow rate of lymph (relative to 0.2% portal vein)

needs to be taken into account when considering the transfer of lutein into blood and lymph from the intestine [24,25]. In addition, we multiplied the amount by each sample volume to calculate the cumulative lymph amount of lutein. There were significant differences between the cumulative amounts up to 24 h after administration in the SMEDDS, SD and powder groups (Fig. 5). Interestingly, the cumulative amount of lutein in the SMEDDS group increased from about 4 h after administration, whereas that in the SD group increased from about 2 h. This difference might be because it takes a longer time to stir with some water and bile and form micelles by peristalsis in the intestinal lumen in the SMEDDS gel, whereas the particles in the SD are smaller and it is easier for them to be dispersed and form micelles. The particle sizes of SMEDDS in the intestinal lumen would be smaller than that of the formulation itself, too.

We previously reported partial involvement of the cholesterol transporter NPC1L1 (Niemann-Pick C1 like-1) and SR-B1 (scavenger receptor class B type 1) in intestinal absorption of lutein [21]. The results of our studies suggested that lutein in the small intestine after oral administration is incorporated into enterocytes via NPC1L1 and SR-B1 or by passive diffusion and then transferred into the lymph stream rather than into the blood stream. Blood should be collected from the portal vein to compare plasma and lymph concentrations of lutein. Further investigations to clarify the transfer of lutein into the blood stream or lymph stream are in progress.

Conclusion

We focused on the lymphatic transport of lutein from the small intestine after oral administration of an SMEDDS and a SD. The lymph concentration of lutein in the SMEDDS group increased gradually from about 4 h after administration, whereas that in the SD group increased from about 2 h. The two formulations of lutein improved lymphatic transport compared with the powder. It was also found that the absorption of lutein after oral administration of each formulation could be clearly evaluated by its cumulative amount in lymph. We consider that our formulations are effective for improvement in the intestinal absorption of lutein.

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Conflict of interest

The authors report no conflicts of interest in this work.

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Figure legends

Fig. 1. Plasma concentrations of lutein after oral administration of powder (A), SMEDDS (B) and SD (C). Lutein (2.5 mg/kg body weight) as each formulation was administered to rats, and blood samples were collected before administration and 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after administration. Each point represents the mean \pm S.D. of 3 measurements.

Fig. 2. Tissue distribution of lutein (liver, kidney, spleen (A) and intestinal mucosa (B)) 4 h after oral administration of each formulation. Lutein (2.5 mg/kg body weight) as each formulation was administered to rats, and tissue samples were collected 4 h after administration. The sample of the small intestine was divided into two segments (upper and lower) and the intestinal mucosa was scraped with a glass slide. The homogenate of each tissue was then prepared. The open column shows the control group. The semi-closed column shows the SMEDDS group and the closed column shows the SD group. *; significantly different at $P < 0.05$ by one-way ANOVA followed by the Tukey-Kramer test. (A) Each column represents the mean with S.D. of 3-5 measurements. (B) Each column represents the mean with S.D. of 3-5 measurements.

Fig. 3. Tissue distribution of lutein (liver, kidney, spleen (A) and intestinal mucosa (B)) 24 h after oral administration of each formulation. Lutein (2.5 mg/kg body weight) as each formulation was administered to thoracic lymph-cannulated rats, and tissue samples were collected 24 h after administration. The sample of the small intestine was divided into two segments (upper and lower) and the intestinal mucosa was scraped with a glass slide. The homogenate of each tissue was then prepared. The open column shows the control group. The semi-closed column shows the SMEDDS group and the closed column shows the SD group. *; significantly different at $P < 0.05$ by one-way ANOVA followed by the Tukey-Kramer test. (A) Each column represents the mean with S.D. of 3-5 measurements. (B) Each column represents the mean with S.D. of 3-5 measurements.

Fig. 4. Lymph concentration profile of lutein up to 24 h after oral administration of each formulation. Lutein (2.5 mg/kg body weight) as each formulation was administered to thoracic lymph-cannulated rats, and lymph was collected up to 24 h after administration. Each sample was cumulative because the flow rate of lymph is very slow. The open column shows the control group. The semi-closed column shows the SMEDDS group and the closed column shows the SD group. Each column represents the mean with S.D. of 3-5 measurements.

Fig. 5. Cumulative lymph amount profile of lutein up to 24 h after oral administration of

each formulation. Lutein (2.5 mg/kg body weight) as each formulation was administered to thoracic lymph-cannulated rats, and lymph was collected up to 24 h after administration. The amount was calculated by multiplying the concentration in Fig. 4 by each sample volume. Open symbols (circles) show the control group. Closed symbols (circles and triangles) show the SMEDDS group and the SD group, respectively. Each point represents the mean with S.D. of 3 measurements. *; significantly different at $P < 0.05$ by one-way ANOVA followed by the Tukey-Kramer test.