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Research paper

# Enhancement of lymphatic transport of lutein by oral administration of a solid dispersion and a self-microemulsifying drug delivery system

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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. These 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.

## 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 pharmaceutics 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

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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 μg/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-1™ (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*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-1™ 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 Section 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 Section 2.3, the average particle sizes (z-average diameter) were measured by using a quasi-elastic light scattering method (Zeta Nano ZS; Malvern Instruments, Herrenberng, 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 s. 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 (1000 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. 1A). 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. 1B). There was a temporarily very high lutein concentration in the SD group (Fig. 1C). 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.